Vol. 99, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 31, 1981 Pages 745-751

PROSTAGLANDIN 9-KETOREDUCTASE AND TYPE II 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE FROM SWINE KIDNEY ARE ALTERNATE ACTIVITIES OF A SINGLE ENZYME PROTEIN

David Guey-Bin Chang, Ming Sun[1] and Hsin-Hsiung Tai[2]

Departments of Chemistry and Biochemistry
North Texas State University and Texas College of Osteopathic Medicine
Denton, Texas 76203

Received February 17, 1981

Summary: Two proteins, each possessing reversible prostaglandin 9-ketoreductase and type II 15-hydroxyprostaglandin dehydrogenase activities, have been purified to homogeneity from swine kidney. Studies on sensitivities to heat inactivation and indomethacin inhibition also indicated that the two different enzyme activities were associated with the same enzyme proteins.

INTRODUCTION

Prostaglandin 9-ketoreductase (9-PGKR) which catalyzes NADPH-linked conversion of prostaglandin E (PGE) to prostaglandin F (PGF) has been shown to be present virtually in every mammalian tissue (1,2). Attempts to purify and to study the regulatory properties of this enzyme have been made in several systems (3-10). The enzyme was found to exist in multiple forms (5,8,9) and was thought to control the PGE/PGF ratio and to couple with $NADP^+$-dependent type II 15-hydroxyprostaglandin dehydrogenase (15-PGDH) in regulating prostaglandin catabolism (4,6). Purification of type II 15-PGDH has also been carried out from several tissues (4,6,8-11). In most instances the partially purified preparation contained both 9-PGKR and 15-PGDH activities although it was not clear whether the reduction of 9-keto function and the oxidation of 15-hydroxyl group were actually catalyzed by the same enzyme protein. We have obtained two homogeneous enzyme proteins from swine kidney and found that both catalyze the reversible oxido-reduction at C-9 and C-15 of prostaglandins. These results, coupled with studies on heat inactivation and indomethacin inhibition, lead us to conclude that previously recognized 9-PGKR and type II 15-PGDH of swine kidney are alternate activities of the same enzyme proteins.

---

[1]Present address: Abbott Laboratories, North Chicago, Illinois 60064
[2]To whom correspondence should be addressed at Department of Biochemistry, NTSU/TCOM, Denton, Texas 76203

0006-291X/81/060745-07$01.00/0

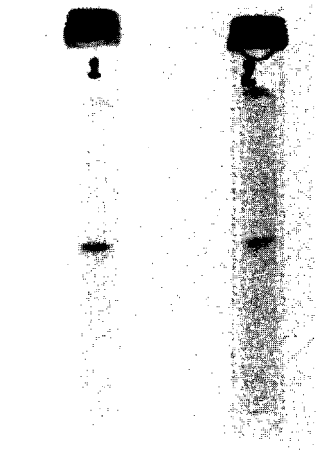
MATERIALS AND METHODS

Materials: Prostaglandins and 15-keto-$PGF_{2\alpha}$ antisera were generously supplied by the Upjohn Company. Ampholine was purchased from Pharmacia Fine Chemical Co.; pyridine nucleotides, TEAE-cellulose, Sephadex gel and indomethacin were obtained from Sigma Chemical Co.

Enzyme Assays: Spectrophotometric method: The 15-PGDH activity was assayed by quantitating the production of 15-keto-PGE_2 from PGE_2 as described by Anggard et al. (12). The assay mixture contained: PGE_2, 56.8 μM; $NADP^+$, 0.2 mM; and enzyme in 1 ml of 0.1M potassium phosphate buffer, pH 7.5. The reaction was allowed to proceed at 37°C for 40 min and then terminated by the addition of 0.1 ml of 2N NaOH. The chromophore generated was quantitated by using a molar extinction coefficient of 27,000 $M^{-1}cm^{-1}$ at 500 nm (13). This method was used for rapid analysis of the chromatographic fractions.

Radioimmunoassay method: The products of 9-PGKR and 15-PGDH catalyzed reactions were quantitated by radioimmunoassay (RIA). The reaction mixture contained: 0.5 mM NADPH or $NADP^+$, 14 μM PGE_2 (for 9-PGKR) or $PGF_{2\alpha}$ (for 15-PGDH) and enzyme in 0.2 ml of 0.1M potassium phosphate buffer, pH 7.5. The reaction was allowed to proceed at 37°C for 10 min and then terminated by boiling for 2 min. After removing denatured proteins by centrifugation the supernatant was diluted and assayed for immunoreactive $PGF_{2\alpha}$ or 15-keto-$PGF_{2\alpha}$. The RIA mixture (0.4 ml) contained: 0.2 ml of unknown sample (or standard), 0.1 ml of diluted antiserum and 0.1 ml of [^{125}I]-labeled prostaglandin tyrosine methyl ester (ca. 10,000 cpm) in RIA buffer, 50mM Tris-HCl, pH 7.5, containing 0.1% gelatin. After one hour of incubation at room temperature, separation of bound from free hapten and quantitation of prostaglandins were done according to Tai and Yuan (14). Specific antisera against $PGF_{2\alpha}$ and 15-keto-$PGF_{2\alpha}$ were produced and characterized as described previously (15). Antisera at 1 to 150,000 and 10,000 final dilution were used for RIA of $PGF_{2\alpha}$ and 15-keto-$PGF_{2\alpha}$ respectively. [^{125}I]-labeled hapten were prepared according to published procedures (14).

Enzyme Purification: Purification was carried out at 4°C. Swine kidneys (500 gms) were homogenized in two volumes of 10 mM Tris-HCl buffer, pH 7.5 containing 1 mM EDTA (Buffer A) in a Waring blender for 2 min. The homogenate was centrifuged at 40,000 xg for 2 min. To the supernatant crude extract was added slowly prechilled acetone (-68°C) to 33% (v/v) with stirring for 10 min. Precipitate was removed by centrifugation at 40,000 xg for 15 min. Acetone was further added to the supernatant to 43% with stirring for 10 min and the precipitate was again removed by centrifugation. Acetone was then added to the final supernatant to 67%. The precipitate obtained was dissolved in 50 ml of Buffer A and designated acetone fraction. This fraction was immediately chromatographed on a Sephadex G-100 column (6 x 100 cm) equilibrated and eluted with Buffer A. The active fractions were pooled and concentrated to 20 ml with an Amicon ultrafiltration PM10 membrane. This fraction was then applied into a TEAE-cellulose column (1.5 x 25 cm) equilibrated with Buffer A. The column was eluted with 10 mM potassium phosphate buffer, pH 7.5 containing 1 mM EDTA (Buffer B). The active fractions were pooled and concentrated to 20 ml by ultrafiltration. This fraction was further purified by isoelectric focusing in a sucrose density gradient using 2.5% ampholine (pH 4-6) in a 110 ml LKB column as described by Vesterberg et al. (16). Water at 3°C was circulated to cool the column. Electrofocusing was performed at 1600 volts for 16 hours. The contents of the column were collected in 1 ml fractions. Two peaks of activity appeared and were designated Form I and II with pI of 5.8 and 4.8 respectively. Active fractions from each peak were respectively pooled, concentrated and passed through a Sephadex G-50 (1 x 15 cm) column equilibrated and eluted with 50 mM potassium phosphate buffer, pH 7.5



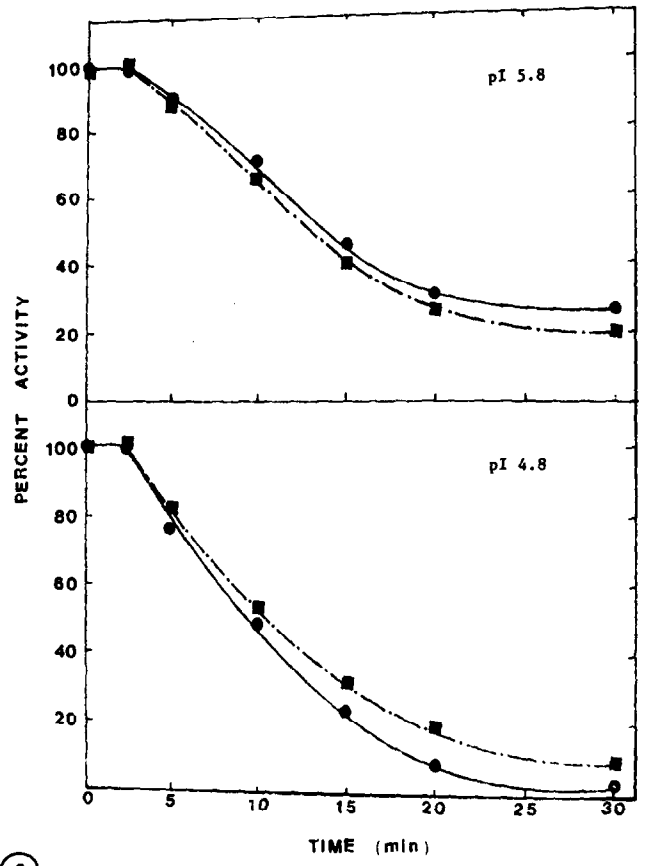


Fig. 1: SDS-polyacrylamide gel electrophoresis of Form I and Form II. Electrophoresis of polyacrylamide gels (7.5%) in the presence of 0.1% SDS were run as described in "Methods". From left to right, Gel 1, Form I (20 µg); Gel 2, Form II (20 µg).

Fig. 2: Effect of heat upon enzyme activities of 15-PGDH and 9-PGKR of Form I (pI 5.8) and Form II (pI 4.8). Enzyme was incubated at 60°C in 0.1M potassium phosphate buffer, pH 7.5. At the indicated time, aliquots were cooled in ice and assayed for the enzyme activities of 9-PGKR (●) and 15-PGDH (■) using RIA Method. A, Form I (55 µg); B, Form II (50 µg).

containing 1 mM EDTA. The active fractions were concentrated by lyophilization and dialyzed against Buffer B for 24 hours. The enzyme was stored in aliquots at -80°.

SDS-Gel Electrophoresis: This was performed according to the method of Weber et al. (17).

Protein Determinations: Estimates of protein concentrations were made by Bradford method using bovine γ-globulin as a standard (18).

RESULTS

Table I summarizes the purification of 9-PGKR and 15-PGDH from swine kidney. The activities of the crude extract could not be accurately measured because of the NADPH oxidase activity and the interference of proteins in RIA procedure. Estimating from the acetone fraction we achieved a hundred fold purification with approximately 30% recovery for each of the two forms. In addition to estimating 9-PGKR and 15-PGDH activities in each fraction, we also determined the activities of the reverse reaction of 9-PGKR and 15-PGDH, namely, 9-PGDH and 15-PGKR catalyzed reactions using $PGF_{2\alpha}$ and 15-keto-$PGF_{2\alpha}$ as substrates and measuring the production of PGE_2 and $PGF_{2\alpha}$ respectively. It was found that the ratio of four different activities remained constant throughout the entire purification procedure. The final preparations of both forms were judged to be homogeneous as evidenced by a single band in SDS-polyacrylamide gel electrophoresis (Fig. 1). Clearly, both enzyme proteins were capable of catalyzing reversible oxido-reduction at C-9 and C-15 of PGE and PGF. Further evidences that the previously recognized 9-PGKR and 15-PGDH activities in swine kidney are associated with the same enzyme protein were derived from heat inactivation and indomethacin inhibition studies. For each protein, the half-lives of 9-PGKR and 15-PGDH activities at 60°C were nearly identical (Fig. 2). However, Form I appeared to have longer half-life than Form II (13.5 min vs. 10 min). The inhibitory effects of indomethacin on the two activities were also closely comparable, although Form I seemed to be more sensitive to inhibition than Form II (I_{50} was 0.4 mM vs. 1 mM) (Fig. 3).

DISCUSSION

Swine renal 9-PGKR and type II 15-PGDH were first partially purified by Lee *et al.* (6). The ratio of 9-PGKR to 15-PGDH during purification was not constant suggesting that the two different activities were associated with two different enzyme proteins. We reported previously that 9-PGKR and 15-PGDH of swine kidney copurified during a series of chromatography steps (9). However, we were not certain whether the two activities resided on the same proteins since the enzyme was not purified to homogeneity. Inability to resolve the two activities from other tissues during a series of chromatography steps was also noted by others (4,6,8,10,11). Nevertheless, Lee and Levine proposed that the coupling of the 9-PGKR and type II 15-PGDH might regulate not only the ratio of PGE_2 to $PGF_{2\alpha}$ but also the duration of their activities (4). In addition to 9-PGKR and type II 15-PGDH activities in swine kidney, Granstrom also found 15-PGKR activity in the cytosol of the same organ (19). Our results indicate that

Table I

Purification of 9-PGKR (I), 9-PGDH (II), 15-PGKR (III) and 15-PGDH (IV) from Swine Kidney

Fraction	Protein (mg)	Enzyme Activity (units)				Specific Activity (units/mg)				Purification (fold)				Recovery (%)			
		I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
I. Crude Extract	9,360																
II. Acetone Fraction (43-67%)	862.5	70	104	24	49	0.08	0.12	0.03	0.06	1	1	1	1	100	100	100	100
III. Sephadex G-100	176.8	83	120	25	55	0.47	0.68	0.14	0.31	5.8	5.7	5	5.5	118	115	104	112
IV. TEAE-Cellulose	71.4	58	82	22	41	0.81	1.15	0.31	0.57	10	9.6	11	10.1	83	79	91	84
Isoelectric Focusing																	
V. Form I (pI 5.8)	2.9	22	33.1	7.7	19.8	7.5	11.3	2.6	6.8	93	94	93	119	31	32	32	40
VI. Form II (pI 4.8)	2.0	18.6	27.4	6.3	15	9.3	13.6	3.1	7.5	115	113	111	132	27	26	26	30

Activities of I and IV were assayed as described in Methods. Activities of II and III were assayed using $PGF_{2\alpha}$ and 15-keto-$PGF_{2\alpha}$ as substrate and following the production of PGE_2 and $PGF_{2\alpha}$ respectively as described for assay of activities of I and IV. One unit of enzyme activity was defined as that amount of enzyme which catalyzed the formation of 1 nmole of product per min under the standard assay conditions.

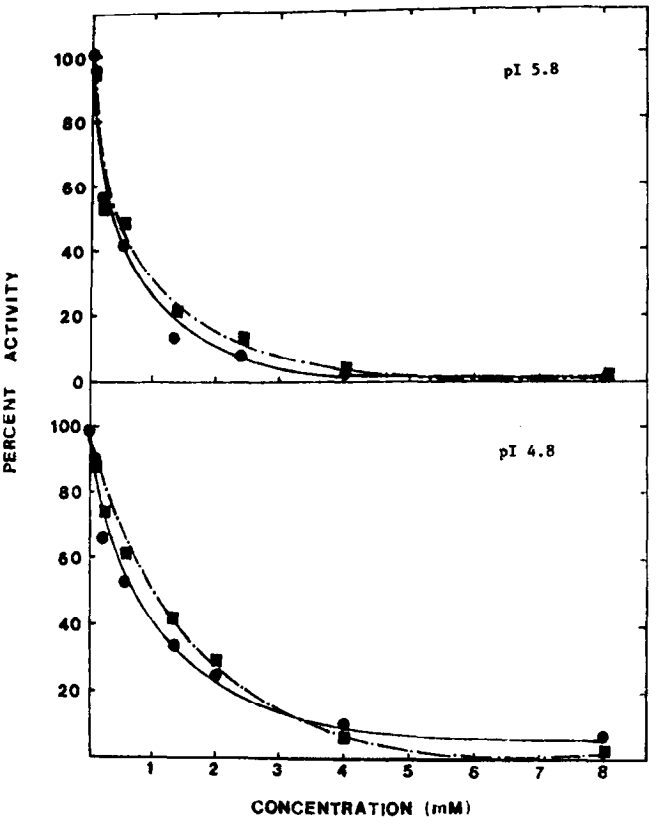


Fig. 3: Inhibition of the enzyme activities of 15-PGDH and 9-PGKR of Form I (pI 5.8) and Form II (pI 4.8) by indomethacin. The enzyme was preincubated in 0.1M potassium phosphate buffer, pH 7.5, with cofactor and inhibitor for 5 minutes before initiating the enzymatic reactions by adding substrates and incubating for another 10 minutes. Enzyme was assayed for activities of 9-PGKR (●) and 15-PGDH (■) using RIA method. A, Form I (55 µg); B, Form II (50 µg).

all three previously recognized activities in swine kidney are actually associated with the same enzyme proteins. The proposition that oxido-reduction at C-9 and C-15 of prostaglandins is essentially catalyzed by a single protein could well be true for every mammalian tissue. Lin and Jarabak also isolated two homogeneous proteins with 9-PGKR and 15-PGDH activities from human placenta (10). However, the use of only two inhibitor concentrations in comparing the degree of inhibition of 9-PGKR and 15-PGDH activities did not provide convincing evidence that a single catalytic site contributed to the two activities observed. Our results demonstrating the closely similar sensitivities of 9-PGKR and 15-PGDH to heat inactivation and indomethacin inhibition suggest that the same catalytic site is responsible for the separate reactions.

An increasing number of possible roles of 9-PGKR and 15-PGDH in physiological systems has been proposed. Weber et al. (20) provided evidence for 9-PGKR as a mediator of salt intake-related prostaglandin-renin interaction. Limas and Limas (21) suggested that 15-PGDH plays a role in the development of hypertension. Ziboh et al. (22) related the increased 9-PGKR activity to onset of the proliferation of the skin induced by essential fatty acid deficiency. In view of the fact that a single protein catalyzes the interconversion of functionally different prostaglandins as well as the inactivation and reactivation of prostaglandins, a careful interpretation of such experimental data is required.

ACKNOWLEDGEMENTS

We thank Dr. J. E. Pike and Dr. K. Kirton of the Upjohn Company for a generous supply of prostaglandins and 15-keto-$PGF_{2\alpha}$ antisera respectively. This work was supported in part by grants from NIH (GM-25247) and AHA (78-865).

REFERENCES

1. Leslie, C. A. and Levine, L. (1973) Biochem. Biophys. Res. Commun. 52:717-724.
2. Lee, S. C. and Levine, L. (1974) J. Biol. Chem. 249:1369-1375.
3. Kaplan, L., Lee, S. C., and Levine, L. (1975) Arch. Biochem. Biophys. 167: 287-293.
4. Lee, S. C. and Levine, L. (1975) J. Biol. Chem. 250:548-552.
5. Lee, S. C. and Levine, L. (1975) J. Biol. Chem. 250:4549-4555.
6. Lee, S. C., Pong, S. S., Katzen, D., Wu, K. Y., and Levine, L. (1975) Biochemistry 14:142-145.
7. Stone, K. J. and Hart, M. (1975) Prostaglandins 10:273-288.
8. Hassid, A. and Levine, L. (1977) Prostaglandins 13:503-515.
9. Sun, M. and Tai, H. H. (1977) Fed. Proc. 36:767.
10. Lin, Y. M. and Jarabak, J. (1978) Biochem. Biophys. Res. Comm. 81:1227-1234.
11. Westbrook, C., Lin, Y.M., and Jarabok, J. (1977) Biochem. Biophys. Res. Commun. 76:943-949.
12. Anggard, E., Larsson, C., and Samuelsson, B. (1971) Acta Physiol. Scand. 81: 396-404.
13. Oliw, E., Lundren, I., and Anggard, E. (1976) Adv. Prostaglandin Thromboxane Res. 1:145-151.
14. Tai, H. H. and Yuan, B. (1978) Anal. Biochem. 87:343-349.
15. Corette, J. C., Harrison, K. L., and Kirton, K. T. (1974) Prostaglandins 5:155-164.
16. Vesterberg, O., Wadstrom, T., Vesterberg, K., Svensson, H., and Malugren, B. (1967) Biochim. Biophys. Acta 133:435-445.
17. Weber, K., Pringle, J. R. and Osborn, M. (1972) Methods Enzymol. 26:3-27.
18. Bradford, M. (1976) Anal. Biochem. 72:248-254.
19. Granström, E. (1971) Biochim. Biophys. Acta 239:120-125.
20. Weber, P., Larson, C., and Scherer, B. (1977) Nature 266:65-66.
21. Limas, C. J. and Limas, C. (1977) Am. J. Physiol. 2(1):H87-H92.
22. Ziboh, V. A., Lord, J. T. and Penneys, N. S. (1977) J. Lipid Res. 18:37-43.