Biochimica et Biophysica Acta, 614 (1980) 1—13

BBA 69016

NAD^+-DEPENDENT 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE FROM PORCINE KIDNEY

I. PURIFICATION AND PARTIAL CHARACTERIZATION

DIANA T.-Y. KUNG-CHAO and HSIN-HSIUNG TAI *

Department of Biochemistry, North Texas State University Health Science Center/Texas College of Osteopathic Medicine, Fort Worth, TX 76107 (U.S.A.)

(Received December 19th, 1979)

Key words: 15-Hydroxyprostaglandin dehydrogenase; NAD^+ dependence; (Porcine kidney)

Summary

The cytoplasmic NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (11α,15-dihydroxy-9-oxoprost-13-enoate:NAD^+ 15-oxidoreductase, EC 1.1.1.141) from porcine kidney was purified to a specific activity of 1.2 unit per mg protein by a series of chromatographic techniques including affinity chromatography.

The native molecular weight of the enzyme was estimated to be 45 000. Substrate specificity studies indicated that the enzyme was NAD^+-specific and was able to catabolize readily various prostaglandins, with the exception of prostaglandin B and thromboxane B. The enzyme was inhibited by sulfhydryl inhibitors, diuretic drugs and various fatty acids.

Introduction

Prostaglandins are rapidly catabolized and inactivated in vivo [1]. The initial step of the catabolism of these biologically active compounds involves the oxidation of 15(S)-hydroxyl group catalyzed by a NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (11α,15-dihydroxy-9-oxoprost-13-enoate:NAD^+ 15-oxidoreductase, EC 1.1.1.141) [2]. This enzyme appears to be the key enzyme in controlling the biological inactivation of prostaglandins, since the 15-ketoprostaglandins possess approx. one tenth or less of the parent compounds' biological activities [3]. Attempts to purify this enzyme from

* To whom correspondence should be addressed at Department of Biochemistry, NTSU/TCOM, P.O. Box 13048, Denton, TX 76203, U.S.A.

various tissues have been made in several laboratories over the past few years [4—11]. However, the enzyme has been purified to homogeneity only from human placenta [11].

Prostaglandins are known to be actively synthesized and catabolized in animal kidneys [12,13]. Prostaglandin A_2 and prostaglandin E_2 have been shown to cause appreciable increases in renal blood flow [14], to stimulate diuresis and natriuresis [15] and to antagonize the action of angiotensin and norepinephrine [16] which are widely regarded as being intimately involved in blood pressure elevation. The fact that 15-hydroxyprostaglandin dehydrogenase is enriched in kidneys of several species [17—19] suggests that this enzyme may play a significant role in regulating renal function. This paper reports purification of 15-hydroxyprostaglandin dehydrogenase from porcine kidney to a highly purified state and investigation of some of its properties. Detailed kinetic studies of this enzyme are presented in the accompanying paper.

Materials and Methods

Dye-reagent concentrate (protein assay), and Affi-Gel blue (100—200 mesh, wet, albumin capacity 13.6 mg/ml) were obtained from Bio-Rad laboratories, Richmond, Ca, U.S.A. Furosemide, acetazolamide, meralluride, and spironolactone were respectively given by Hoechst Pharmaceuticals, Inc., Somerville, NJ, U.S.A., Lederle Laboratories, Pearl River, NY, U.S.A., Lakeside Laboratories, Milwaukee, WI, U.S.A., and Searle Co., Chicago, IL, U.S.A. Chlorothiazide, hydrochlorothiazide, and ethacrynic acid were obtained from Merck, Sharp and Dohme Research Laboratories, Rahway, NJ, U.S.A. 5′-AMP-Sepharose was obtained from the Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A. Agarose-hexane-NAD^+ (AGNAD Type 1, 3, 4) were purchased from the P.O. Biochemicals, Milwaukee, WI, U.S.A. *p*-Hydroxymercuribenzoate, 5,5′-dithiobis(2-nitrobenzoic acid), iodoacetic acid (recrystallized from petroleum ether prior to use), sodium mersalyl, *N*-ethylmaleimide, sodium pyruvate, blue dextran, horse heart cytochrome *c*, rabbit muscle lactate dehydrogenase (L-lactate:NAD^+ oxidoreductase EC 1.1.1.27, 750 U/mg), horse liver alcohol dehydrogenase (alcohol:NAD^+ oxidoreductase EC 1.1.1.1, 1.61 U/mg), trypsin inhibitor, bovine serum albumin, soybean lipoxidase (linoleate:oxygen oxidoreductase EC 1.13.11.12, 165 000 U/mg), arachidonic acid, linoleic acid, oleic acid, NAD^+, NADH, 3-acetylpyridine adenine dinucleotide, thionicotinamide-NAD^+, nicotinamide hypoxanthine dinucleotide, 3-pyridinealdehyde-NAD^+, DL-dithiothreitol, DEAE-cellulose (exchange capacity 0.9 meq/gm), Sephadex G-25 and G-100 were obtained from the Sigma Chemical Co, St. Louis, MO, U.S.A., Ultrapure $(NH_4)_2SO_4$ was obtained from Schwarz/Mann Co., Orangeburg, NY, U.S.A. Stearic acid and palmitic acid were obtained from Supelco, In., Bellefonte, PN, U.S.A. Prostaglandin E_1, prostaglandin E_2, prostaglandin A_1, prostaglandin A_2, prostaglandin $F_{1\alpha}$, prostaglandin $F_{2\alpha}$, prostaglandin I_2, prostaglandin B_2, 15-keto-prostaglandin E_2, 6-keto-prostaglandin $F_{1\alpha}$, and thromboxane B_2 were kind gifts from Dr. John Pike of the Upjohn Co., Kalamazoo, MI, U.S.A.

Enzyme assays

Two different enzyme assays for 15-hydroxyprostaglandin dehydrogenase were employed.

Method A. The assay was based on the development of a strong and transient chromophore at 500 nm following alkali treatment of the reaction product 15-keto-prostaglandin E_2 as described by Anggard and Samuelsson [4]. The assay mixture contained 0.5 μmol NAD^+, 28 nmol prostaglandin E_2, and enzyme in a final volume 1 ml of 50 mM potassium phosphate buffer (pH 7.5). The reaction was carried out at 37°C and terminated by the addition of 0.1 ml of 2 M NaOH. The absorbance of the chromophore was determined at 500 nm, and the concentration of 15-keto-prostaglandin E_2 was estimated using a molar extinction coefficient of 27 000 [20]. This assay was used for rapid analysis of chromatographic fractions. One unit (U) of enzyme activity is defined as the amount of enzyme which catalyzes the formation of 1 μmol of 15-keto-prostaglandin E_2 from prostaglandin E_2 in 1 min under the above conditions.

Method B. Enzyme activity was determined by following the formation of NADH fluorometrically. The reaction mixture contained 0.5 μmol NAD^+, 28 nmol prostaglandin E_1, and enzyme in a final volume of 1 ml of 50 mM potassium phosphate buffer (pH 7.5), containing 0.02% bovine serum albumin and 1 mM EDTA. The reaction was initiated by the addition of enzyme and allowed to proceed at room temperature. The NADH formed in the reaction mixture was recorded by the increase in fluorescence at 460 nm with excitation at 340 nm, using Aminco SPF-500 coupled to a Aminco *X—Y* recorder. This instrument was standardized using different amounts of NADH determined by direct measurements of the absorbance at 340 nm, using a molar extinction coefficient of $6.22 \cdot 10^3\ M^{-1} \cdot cm^{-1}$ [21]. All the substrate specificity studies and the effect of sulfhydryl reagents and diuretic drugs were carried out by this method.

Protein determination

Protein was determined by the dye-binding assay of Bradford [22], using bovine serum albumin as a standard.

Molecular weight determination

Sephadex G-100 gel was swollen and equilibrated in 50 mM potassium phosphate buffer (pH 7.5), containing 1 mM EDTA, 0.2 mM dithiothreitol, and 0.25% sucrose, and packed under gravity. Even packing of each column was checked by watching the passage through it of a band of colored substance (e.g. blue dextran). The Sephadex G-100 column (2.7 × 38.5 cm) was washed with equilibrating buffer overnight at 4°C until stabilization of bed height and a constant flow rate of 30 ml/h were achieved. The void volume (V_0) was determined with blue dextran (M_r 2 000 000). Proteins of known molecular weights, namely cytochrome *c*, trypsin inhibitor, bovine serum albumin, horse liver alcohol dehydrogenase, soybean lipoxidase, and rabbit muscle lactic dehydrogenase were used to calibrate the column. Bovine serum albumin was detected by absorbance at 280 nm. Trypsin inhibitor was measured by absorbance at 215 nm. Cytochrome *c* was estimate at 412 nm. For alcohol dehydrogenase assay, a mixture containing 2 M ethanol/0.5 mM NAD^+ in 0.1 M Tris-HCl

(pH 8.0) containing 1 mM EDTA, and sufficient alcohol dehydrogenase to effect an absorbance change at 340 nm was used. For lactate dehydrogenase assay, the mixture contained 2 mM NAD^+, 5 mM sodium pyruvate in 0.1 M potassium phosphate buffer (pH 7.5) containing 1 mM EDTA, and enzyme. For lipoxidase assay, the mixture contained 0.6 mM linoleate and enzyme in 0.2 M borate buffer (pH 9.0). The increased absorption accompanying linoleate oxidation was measured at 234 nm. All these enzyme assays were carried out at 25°C.

Purification of NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase

All steps of purification were carried out at 0—4°C. Porcine kidneys were obtained fresh from a local slaughterhouse and transported to the laboratory in ice. Kidneys were ejther used immediately or frozen at —80°C for later use. All the buffers included 0.2 mM dithiothreitol to protect the enzyme from inactivation.

A. Preparation of crude extract. Porcine kidneys (198 g) were cut into small pieces, and homogenized in 2 vols. of 50 mM potassium phosphate buffer (pH 7.5), containing 0.2 mM dithiothreitol, and 1 mM EDTA, in a Waring blendor for three 1 min periods separated by cooling. The homogenate was centrifuged at 34 000 × *g* for 25 min in a Sorvall refrigerated centrifuge. The supernatant was referred to as Fraction I.

B. Ammonium sulfate fractionation. To the clear supernatant was added 176 g of $(NH_4)_2SO_4$ per l to reach 30% saturation with constant stirring for 1 h. The pH was kept near 7.5 by the addition of 3.0 M NH_4OH. The precipitate was removed by centrifuge at 12 000 × *g* for 20 min after stirring for 1 h. The clear supernatant was then brought to 55% saturation by adding 162 g $(NH_4)_2SO_4$ per l with stirring for another h. The pH of this solution was adjusted to 7.5. The precipitate collected by centrifugation at 12 000 × *g* for 20 min was dissolved in 116 ml of 10 mM potassium phosphate buffer (pH 7.5), containing 0.2 mM dithiothreitol and 1 mM EDTA (Buffer A). This solution was referred to as Fraction II.

C. DEAE-cellulose chromatography. Fraction II was desalted through a Sephadex G-25 column (5 × 51.0 cm) which had been equilibrated with Buffer A. The desalted fraction (265 ml) was loaded onto a DEAE-cellulose column (5 × 22.5 cm) equilibrated with Buffer A. The column was washed with 30 ml potassium phosphate buffer (pH 7.5), containing 0.2 mM dithiothreitol and 1 mM EDTA, until the A_{280} of the eluate was near 0.09. Elution was started with a linear gradient containing 700 ml of 30 mM KCl in 10 mM potassium phosphate buffer (pH 7.5), and 700 ml of 600 mM KCl in the same buffer. The enzyme was eluted around 55 mM KCl. The peak activity was eluted around 0.2 M KCl. Fractions which contained enzyme activity were pooled and concentrated with 472 g ammonium sulfate per l in order to reach 70% saturation. The mixture was stirred for 1 h and centrifuged at 12 000 × *g* for 20 min. The precipitate was dissolved in 42 ml of Buffer A. This solution is referred to as Fraction III. The chromatographic profile is shown in Fig. 1.

D. Sephadex G-100 filtration. Fraction III was applied to a Sephadex G-100 column (5 × 102 cm) equilibrated with Buffer A. Elution was carried out with the same buffer. Active fractions were pooled and concentrated to 47 ml by

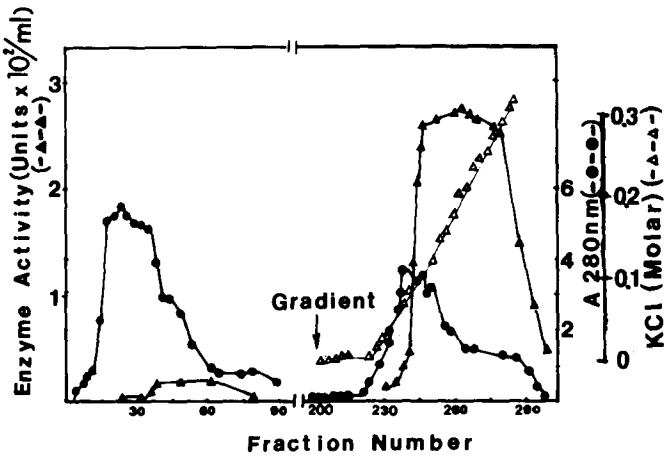

Fig. 1. Chromatography of 15-hydroxyprostaglandin dehydrogenase on DEAE-cellulose column. Protein concentration was determined by absorption at 280 nm. The enzyme was assayed according to Method A. The concentrations of KCl during gradient elution were determined by conductivity measurement using Markson's ElectroMark Analyzer, which had been standardized with conductivity standards. After absorption at 280 nm reached 0.09 by washing with 3 l of equilibrating buffer, a linear KCl gradient was initiated as indicated. Fraction volume, 14.1 ml; flow rate, 130 ml/h.

Amicon Ultrafiltration cells, using a 10 000 molecular weight cut-off membrane (PM-10). This fraction was designated Fraction IV. A typical activity profile is shown in Fig. 2.

E. Affi-gel blue affinity chromatography. The Affi-gel blue colum (1.4 X 38

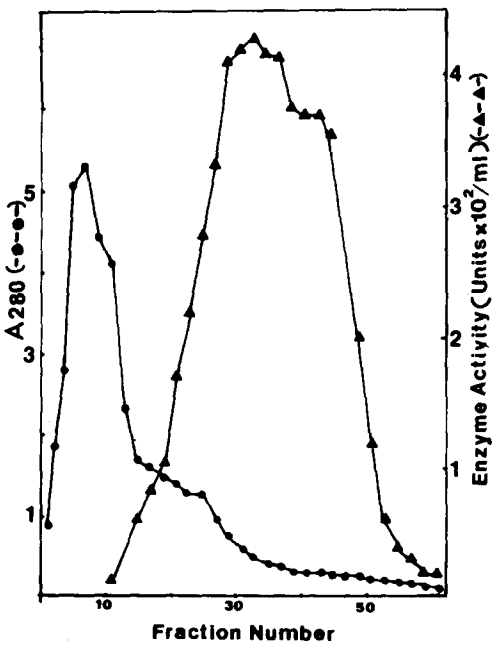

Fig. 2. Chromatography of 15-hydroxyprostaglandin dehydrogenase on Sephadex G-100 column. Fractions were collected as soon as protein began to appear in the eluate. The protein concentrations were determined by absorption at 280 nm. The enzyme was assayed according to Method A. Fraction volume, 14.5 ml; flow rate, 100 ml/h.

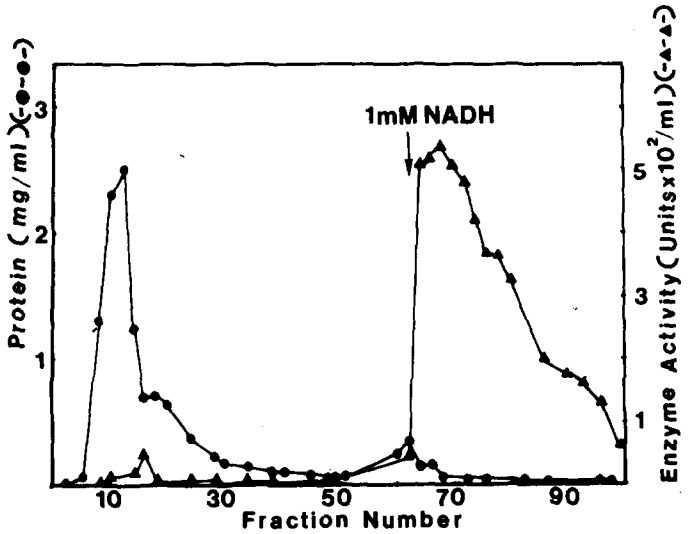

Fig. 3. Chromatography of 15-hydroxyprostaglandin dehydrogenase on Affi-Gel Blue affinity column. Fraction of 7.5 ml were collected at a flow rate of 50 ml/h. Protein concentrations were determined by absorbance at 280 nm before using 1 mM NADH to elute the enzyme. Protein concentrations were measured by Bradford dye reagent after elution with NADH. Enzyme activities were assayed by Method A.

cm) was packed under gravity and equilibrated with Buffer A. Fraction IV was applied to this column, and the column was then washed with the same buffer until the A_{280} reading was near 0.1. 15-Hydroxyprostaglandin dehydrogenase was eluted from the column by using Buffer A containing 1 mM NADH. The result is shown in Fig. 3. Active fractions were pooled and concentrated immediately to 15.3 ml by using Amicon Ultrafiltration cells (with 10 000 M_r cut-off membrane). The final partially purified enzyme (Fraction V) was stored in small aliquots at −80°C. This single step removed 95% of the protein from the last step and increased purification 14.5-fold. Protein concentration (47 μg/ml) was low in NADH eluate. Inactivation can be minimized by concentration of this fraction using Amicon Ultrafiltration cells. All the studies on chemical properties and kinetic mechanism were carried out by this fraction.

Results

Table I summarizes the steps for the purification of porcine kidney 15-hydroxyprostaglandin dehydrogenase. The final preparation showed a 377-fold enrichment with an overall recovery of activity of 51% under the standard assay of Method A. The specific activity was 1236 mU per mg.

Physicochemical properties

A. Molecular weight determination. Molecular weight determination of 15-hydroxyprostaglandin dehydrogenase from porcine kidneys was performed by Sephadex G-100 gel filtration. When the elution volume of 15-hydroxyprostaglandin dehydrogenase was correlated with the elution volumes of proteins of known molecular weights [23], an apparent molecular weight of

TABLE I

PURIFICATION OF 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE FROM PORCINE KIDNEY

Fraction		Volume (ml)	Total protein (mg)	Total activity (units)	Specific activity (units/mg) ($\times 10^3$)	Purification (-fold)	Percentage recovery
I	Crude extract	400	9440	30.9	3.28	—	100
II	Ammonium sulfate precipitate (30—55%)	116	4800	27.0	5.80	1.78	90.3
III	DEAE-cellulose	822	1070	28.0	26.20	8.00	90.6
IV	G-100	48	310	26.0	85.00	27.20	84.0
V	Affi-gel Blue concentrate	15.3	12.7	15.7	1236.00	377.00	50.8

45 000 was estimated.

B. Substrate specificity. 15-Hydroxyprostaglandin dehydrogenase was examined for its ability to oxidize a number of prostaglandins. The results are summarized in Table II. Nearly all prostaglandins, with the exception of prostaglandin B_2, were metabolized by the enzyme. K_m values in increasing order were prostaglandins E's, A's, I_2, 6-keto-prostaglandin $F_{1\alpha}$, F's and thromboxane B_2. Both prostaglandin E's and prostaglandin A's had K_m values in the range of 10^{-6} M, while other prostaglandins had K_m values in the order of 10^{-5} M. Thromboxane B_2 was a very poor substrate for the enzyme. Since prostaglandin E_2 was found to be the best substrate, it was used in all subsequent assays.

C. Coenzyme specificity. NAD^+, $NADP^+$ and a number of NAD^+ analogs were examined for their capability to serve as coenzyme for 15-hydroxyprostaglandin dehydrogenase. Table III shows the activity of 15-hydroxyprosta-

TABLE II

SUBSTRATE SPECIFICITY OF PORCINE RENAL 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE

All the prostaglandins were respectively dissolved in ethanol at the concentration of 1 mg/ml and diluted in assay buffer before experiment. Prostaglandin-I_2 was made fresh. The assay was done by Method B, using 4 μg of enzyme. The concentration of prostaglandin substrate was varied and the concentration of NAD^+ was kept at 0.5 mM. K_m values were determined by the respective double reciprocal plots. PG. postaglandin; TX, thromboxane.

Prostaglandins	K_m (μM)
PGE_1	1.2
PGE_2	1.2
PGA_1	1.7
PGA_2	4.6
$PGF_{1\alpha}$	19.2
$PGF_{2\alpha}$	12.5
PGI_2	5.0
6-Keto-$PGF_{1\alpha}$	5.1
TXB_2	400.0
PGB_2	Inactive

TABLE III

COENZYME SPECIFICITY OF PORCINE RENAL 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE

The enzyme (6 μg) was assayed in the presence of 1 mM of NAD^+ or its analogs by Method B.

Coenzymes	Activity (pmol/min)
NAD^+	220
3-Acetylpyridine adenine dinucleotide	38.5
$NADP^+$	20
Nicotinamide hypoxanthine dinucleotide	7
Thionicotinamide-NAD^+	0
3-Pyridinealdehyde-NAD^+	0
Deamino-NAD^+	17

glandin dehydrogenase in the presence of each nucleotide at 1 mM. NAD^+ showed the best activity. $NADP^+$ exhibited only one tenth of NAD^+ activity. Among other NAD^+ analogs tested, only 3-acetypyridine adenine dinucleotide and deamino-NAD^+ showed some coenzyme activity.

Inhibitor studies

A. Effect of sulfhydryl inhibitors. Five different sulfhydryl inhibitors, 5,5'-dithiobis(2-nitrobenzoic acid), *N*-ethylmaleimide, *p*-hydroxymercuribenzoate, sodium mersalyl and iodoacetic acid were tested for their effects on 15-hydroxyprostaglandin dehydrogenase. The results are indicated in Table IV. It is apparent that dithiobisnitrobenzoic acid is the most potent inhibitor and iodoacetic acid is the least effective.

B. Effect of diuretic drugs. Since prostaglandin E_2 and prostaglandin A_2 have been shown to have some diuretic activity, and 15-hydroxyprostaglandin dehydrogenase greatly reduces that activity, a series of known diuretic compounds was tested for their ability to inhibit the enzyme, as shown in Table V. The organomercurial diuretics, meralluride and sodium merasalyl, were found to be potent inhibitors of 15-hydroxyprostaglandin dehydrogenase with I_{50} of 3.3 and 1.5 μM, respectively. The loop diuretics — furosemide, a sulfonamide derivative, and ethacrynic acid, an aryloxyacetic acid derivative — are also good inhibitors of 15-hydroxyprostaglandin dehydrogenase, with an

TABLE IV

EFFECT OF SULFHYDRYL INHIBITORS ON PORCINE RENAL 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE ACTIVITY

I_{50} is defined as the concentration of inhibitor required to produce 50% inhibition of enzyme activity under the standard assay conditions of Method B. The concentration of the compounds listed varied from 0 to 1 mM. Enzyme activity at zero concentration was taken as 100%. 5 μg of enzyme was used.

Additions	I_{50} (μM)
Sodium mersalyl	1.5
p-Hydroxymercuribenzoate	2.5
N-Ethylmaleimide	36.0
Dithiobisnitrobenzoic acid	0.9
Iodoacetic acid	650.0

TABLE V

EFFECT OF DIURETIC DRUGS ON PORCINE RENAL 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE

Methods of determining I_{50} and conditions of enzyme assay were the same as those stated in Table IV.

Additions	I_{50} (μM)
Sodium mersalyl	1.5
Meralluride	3.3
Ethacyrnic acid	109.0
Furosemide	91.0
Chlorothiazide	240.0
Hydrochlorothiazide	1030.0
Acetazolamide	>1000.0
Spironolactone	277.0

I_{50} of 91 and 109 μM. Thiazide diuretics such as chlorothiazide and hydrochlorothiazide, and the aldosterone antagonist, spironolactone, are relatively weak inhibitors. Acetolamide, a carbonic anhydrase inhibitors, was not inhibitory at the concentrations studied.

C. Effect of fatty acids. A number of saturated and unsaturated fatty acids were investigated for their effects on 15-hydroxyprostaglandin dehydrogenase. Oleic acid, linoleic acid, arachidonic acid, palmitic acid and stearic acid were found to be inhibitory to the enzyme at low μM range, as shown in Table VI. Oleic acid, the most potent inhibitor, has a K_i of 1.25 μM. Inhibition as examined by Dixon plot was of non-competitive type.

Discussion

Porcine kidney was previously shown to be a rich source of 15-hydroxyprostaglandin dehydrogenase [17]. Attempts to purify this enzyme by classical chromatographic techniques alone failed to obtain a preparation of sufficiently high specific activity with good recovery. Efforts to purify this enzyme were directed to the used of affinity chromatographic techniques. Several types of NAD^+-agarose, having agarose derivatized to attach to different regions of the nucleotide, were tested for possible affinity purification of porcine renal 15-hydroxyprostaglandin dehydrogenase. None of these affinity columns was

TABLE VI

EFFECT OF FATTY ACIDS ON PORCINE RENAL 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE

Fatty acids were dissolved in ethanol and diluted in assay buffer before experiment. The assay was done by Method B using 5 μg of enzyme. Fatty acid concentrations were varied at two fixed concentrations of prostaglandin E_1, 5.6 and 28.3 μM. The Dixon plot was used to estimate K_I.

Fatty acids	K_I (μM)
Oleic acid	1.25
Linoleic acid	4.10
Arachidonic acid	6.00
Palmittic acid	4.00
Stearic acid	40.00

able to specifically retain porcine renal enzyme. This is in contrast to human placental 15-hydroxyprostaglandin dehydrogenase, which could be specifically purified by NAD^+-hexane-agarose [11]. 5'-AMP-Sepharose, which has been shown to be an effective affinity gel for the purification of a number of dehydrogenases [24], also failed to bind porcine renal enzyme. Prostaglandin $F_{2\alpha}$-aminohexyl-Sepharose was also proven to be of little value although it was reported to be useful for purifying bovine lung and porcine kidney 15-hydroxyprostaglandin dehydrogenase [6,20]. The binding of 15-hydroxyprostaglandin dehydrogenase to prostaglandin-Sepharose column reported in the latter two cases was probably due to nonspecific binding, since the enzyme could not be eluted with prostaglandins and could be eluted only with acetate buffer at pH 4.0. The recovery of activity was also low with this acidic elution. The only affinity gel which was found to specifically bind porcine renal enzyme was Affi-Gel Blue, or Blue Sepharose. This type of affinity gel has been increasingly used to purify enzyme having dinucleotide fold [25]. Porcine renal 15-hydroxyprostaglandin dehydrogenase was found to bind tightly to the Cibacron blue dye. Elution of the enzyme could not be effected even with 10 mM NAD^+ unless 1 mM NADH was added in the buffer. This procedure resulted in efficient purification in one step. Although the specific activity of the purified enzyme was found to be lower than that of homogeneous enzyme from human placenta [11] (1.2 U/mg vs. 1.7 U/mg), it was higher than those reported from porcine lung [4], bovine lung [5,6] and porcine kidney [8]. Further efforts to purity it to homogeneity by preparative electrophoresis and isoelectric focusing have so far failed partly because of the unstable nature of the enzyme. However, the highly purified enzyme was devoid of any other enzymes involved in prostaglandin metabolism, namely, 9-keto-prostaglandin reductase [26], $NADP^+$-dependent 15-hydroxyprostaglandin dehydrogenase [27] and Δ^{13}-15-keto-prostaglandin reductase [17]. It was of sufficient purity to carry out some aspects of characterization and kinetic studies. A summary of the partial purification of the enzyme from porcine kidney is shown in Table I. Near quantitative recovery of enzyme activity in early steps may indicate that some endogenous inhibitors were present in the crude preparation which were gradually removed during purification, but it may as well reflect a contamination with Δ^{13}-15-keto-prostaglandin reductase, which catalyzes further the reduction of Δ^{13}-double bond, resulting in the underestimation of the enzyme activity in early fractions.

The molecular weight of 15-hydroxyprostaglandin dehydrogenase from porcine kidney was estimated to be 45 000. It is comparable to those from bovine lung, 40 000 [27] and human placenta, 51 500 [11]. All these values were estimated by Sephadex gel filtration.

15-Hydroxyprostaglandin dehydrogenase from porcine kidney appeared to have lower K_m values for the same prostaglandins than those from other sources [4,9,29]. The difference may be caused by differences in the incubation conditions, the purity, and the source of the enzyme. It is worth nothing that prostaglandin I_2 and its stable hydrolyzed product, 6-keto-prostaglandin $F_{1\alpha}$, are good substrates for porcine renal 15-hydroxyprostaglandin dehydrogenase. This finding is consistent with the recent reports that prostaglandin I_2 and 6-keto-prostaglandin $F_{1\alpha}$ are rapidly metabolized to 6,15-diketo-13,14-

dihydro-prostaglandin $F_{1\alpha}$ by rabbit and rat kidneys [30,31]. The fact that thromboxane B_2 is a poor substrate suggests that the enzyme may require a cyclopentane ring in addition to the presence of a 15(S)-hydroxyl group. However, it should be emphasized that these results are from in vitro data. Other factors may influence the metabolic fate of these compounds when administered to the intact animal. A good example is that prostaglandin As circulate through the lung without appreciable loss of biological activity [32] despite the fact that they are rapidly oxidized by purified lung 15-hydroxyprostaglandin dehydrogenase [29].

Studies of the structural requirements for binding to NAD^+ site indicated that any modification of the NAD^+ molecule results in substantial loss of coenzyme activity. Alteration of the carboxamide group of the nicotinamide moiety appears to affect more of the coenzyme capacity than changing in the adenosine portion. For instance, NAD^+ analogs modified in the carboxamide group, such as thionicotinamide-NAD^+ and 3-pyridinealdehyde, could not serve as coenzyme. On the other hand, analogs modified in the adenosine portion, such as $NADP^+$ and deamino-NAD^+, showed some coenzyme activity. This finding is in contrast with the observation reported for porcine lung 15-hydroxyprostaglandin dehydrogenase [33]. The difference in the source and the purity of the enzyme may account for the discrepancy. Selective control of the enzyme activity through the NAD^+ site had limited pharmacological potential, because of the presence of a similar site on numerous other dehydrogenases, Nevertheless, a study of the NAD^+ site, which is an essential feature of the enzyme, contributes to a better understanding of how 15-hydroxyprostaglandin dehydrogenase interacts with the coenzyme.

Virtually all sulfhydryl inhibitors except iodoacetic acid were found to be potent inhibitors of porcine renal enzyme. This suggests that certain sulfhydryl groups of the enzyme are crucial to the catalytic function. The fact that the enzyme is very labile in the absence of sulfhydryl compound in the buffer is consistent with this observation.

The effects of saturated and unsaturated fatty acids on porcine renal 15-hydroxyprostaglandin dehydrogenase were very similar to those reported for procine lung 15-hydroxyprostaglandin dehydrogenase [33]. Fatty acids, saturated or unsaturated, are apparently potent inhibitors of the enzyme. The significance of this inhibition is not clear. However, it is known that free fatty acids are present at low concentrations intracellularly. They are released in high concentrations upon humoral or other stimulations. The released arachidonic acid is rapidly converted to prostaglandins and the related biologically active compounds. It is possible that the free fatty acids released along with arachidonic acid serve as endogenous modulators to regulate the half-life of prostaglandins.

The effects of diuretic drugs on procine renal 15-hydroxyprostaglandin dehydrogenase are particularily interesting. The results seem to suggest a slight correlation between the inhibitory potency and diuretic efficacy. Mercurial diuretics are among the most potent diuretics. Mercury is presumed to inhibit enzymes involved in the reabsorption of sodium in the proximal tubules, by combination with sulfhydryl groups of the enzyme. According to the efficacy of diuresis, mercurials produce a 20—25% loss in filtered sodium in the human

system, Ethacrynic acid and furosemide produce a 25—35% loss in filtered sodium. These drugs exert their diuretic effects by blocking active chloride transport in the ascending limb of Henle's loop, probably in the form of a complex [34]. The thiazides produce a 10—15% loss in filtered sodium, and act primarily on the early distal tubule (cortical diluting segment) and on the sodium transporting mechanism. Spironolactone is structurally related to the natural hormone, aldosterone, and acts by competing with aldosterone for its target site in the distal tubule. It will produce a 3% loss of filtered sodium as a diuretic; however, it is primarily used for its potassium-sparing abilities. The present results suggest some correlation between inhibition of 15-hydroxyprostaglandin dehydrogenase and diuretic efficacy. Mercurial diuretics are indeed the most potent inhibitors of porcine renal 15-hydroxyprostaglandin dehydrogenase among all diuretics studied. Furosemide and ethacrynic acid are also good inhibitors of the enzyme. Thiazides and spironolactone are relatively weak inhibitors of the enzymes, as shown by higher I_{50} values. Similar findings were previously reported for 15-hydroxyprostaglandin dehydrogenase from human placenta [35].

Acknowledgement

We are indebted to Dr. John Pike of the Upjohn Company for his kind gifts of prostaglandins. This study was supported in part by grants from U.S. Public Health Servuce (GM-25247) and American Heart Association (78-865).

References

1 Samuelsson, B., Granström, E., Green, K. and Hamberg, M. (1971) Ann. N.Y. Acad. Sci. 180, 138—163
2 Anggård, E. and Samuelsson, B. (1964) J. Biol. Chem. 239, 4097—4102
3 Anggard, E. (1966) Acta Physiol. Scand. 66, 509—510
4 Anggard, E. and Samuelsson, B. (1966) Ark. Kem. 25, 293—340
5 Matschinsky, F.M., Shanahan, D. and Ellerman, J. (1974) Anal. Biochem. 60, 188—199
6 Nagasawa, M., Chau, J.A. and Sih, C.J. (1974) Prostaglandins 8, 221—230
7 Sun, F., Armour, S.B., Bockstons, V.R. and McGuire, J.C. (1976) Ark. Prostaglandin Thromboxane Res. 163—169
8 Tai, H.H., Tai, C.L. and Hollander, C.S. (1974) Biochem. Biophys. Res. Commun. 57, 457—462
9 Thaler-Dao, H., Saintot, M., Baudin, G. Descomps, B. and Gastes de Paulet, A. (1974) FEBS Lett. 48, 204—208
10 Jung, A., Schlegel, W., Jackish, R., Friedrich, E.J., Wendel, A. and Rückrich, M.F. (1975) Hoppe-Seylers Z. Physiol. Chem. 356, 787—789
11 Braithwaite, S.S. and Jarabak, J. (1975) J. Biol. Chem. 250, 2315—2318
12 Tai, H.H., Tai, C.L. and Hollander, C.S. (1976) Biochem. J. 154, 257—264
13 Granström, E. (1971) Biochim. Biophys. Acta 239, 120—125
14 Herbaczynska-Cedro, K. and Vane, J.R. (1973) Circulation Res. 33, 428—436
15 Stanhoy, J.R., Ott, C.E. and Schneider, E.G. (1974) Am. J. Physiol. 226, 1015—1022
16 Aiken, J.W. and Vane, J.R. (1973) J. Pharmacol. Exptl. Therap. 184, 678—687
17 Anggard, E., Larsson, C. and Samuelsson, B. (1971) Acta. Physiol. Scand. 81, 396—404
18 Tai, H.H. (1976) Biochemistry 15, 4586—4592
19 Moore, P.K. and Hoult, J.R.S. (1977) Biochim. Biophys. Acta 528, 276—287
20 Oliw, E., Lunder, I. and Anggard, E. (1976) Adv. Prostaglandin Thromboxane Res. 1, 147—151
21 Horecker, B.L. and Kornberg, A. (1949) J. Biol. Chem. 175, 385—390
22 Bradford, M. (1976) Anal. Biochem. 72, 248
23 Andrews, P. (1964) Biochem. J. 91, 222—233
24 Guilford, H., Larsson, P.O. and Mosbach, K. (1972) Chem. Scripta 2, 165—170
25 Thompson, S.T., Cass, K.H. and Stellwagen, E. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 669—672

26 Lesile, C.A. and Levine, L. (1973) Biochem. Biophys. Res. Commun. 52, 717—724
27 Lee, S.C. and Levine, L. (1975) J. Biol. Chem. 250, 548—552
28 Hansen, H.S. (1974) Prostaglandins 8, 95—105
29 Nakano, J., Anggard, E. and Samuelsson, B. (1969) Eur. J. Biochem. 11, 386—389
30 Wong, P.Y.-K., McGiff, J.C., Cagen, L., Malik, K.U. and Sun, F. (1979) J. Biol. Chem. 254, 12—14
31 Pace-Asciak, C.R., Carrara, M.C. and Domazet, Z. (1977) Biochem. Biophys. Res. Commun. 78, 115–121
32 Piper, P.J., Vane, J.R. and Wyllie, J.H. (1970) Nature 225, 600—604
33 Marrazzi, M.A. and Matschinsky, F.M. (1972) Prostaglandins 1, 373—388
34 Burg, M. and Green, N. (1973) Kidney Int. 4, 301—308
35 Paulsrud, J.G., Miller, O.N. and Schlegel, W. (1974) Fed. Proc. 33, 590