NADP-LINKED 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE FROM HUMAN PLACENTA: PARTIAL PURIFICATION AND CHARACTERIZATION OF THE ENZYME AND IDENTIFICATION OF AN INHIBITOR IN PLACENTAL TISSUE

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SUMMARY: An NADP-linked 15-hydroxyprostaglandin dehydrogenase has been identified in human placental tissue and partially purified. Prostaglandins of the A and B series are good substrates for this enzyme while those of the E and F series are not. This enzymic preparation also catalyzes oxido-reductions at the 9 position of the prostaglandin molecule; these are slow compared to those occurring at the 15 position of the prostaglandins in the A and B series. Disc gel electrophoresis of the purified enzyme reveals the presence of three protein bands which contain dehydrogenase activity. Boiled placental homogenates contain an inhibitor which appears to be specific for the NADP-linked 15-hydroxyprostaglandin dehydrogenase. The inhibitor is heat stable and has a molecular weight of 6,000 - 7,000.

INTRODUCTION

The biological inactivation of prostaglandins of the E and F series has been shown to proceed through the following pathway: there is first oxidation of the 15-hydroxyl group to a ketone, followed by reduction of the 13, 14-double bond (1). We have recently demonstrated the existence of this pathway in human placenta and have purified and characterized the placental enzymes which catalyze these transformations, 15-hydroxyprostaglandin (NAD) dehydrogenase (2,3) and 15-ketoprostaglandin Δ^{13} reductase (4).

The present report describes the identification and partial purification of a placental 15-hydroxyprostaglandin (NADP) dehydrogenase for which prostaglandins of the A and B series are good substrates. To our knowledge, this is the first report of an enzyme which can oxidize PGB_1. Evidence is also provided for the existence of isoenzymes with this

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activity and for the presence of an inhibitor of this activity in placental homogenates.

MATERIALS AND METHODS

Materials. All of the prostaglandins used were generously provided by the Upjohn Company, Cibacron Blue 3G-A was a gift from Ciba-Geigy, and pyridine nucleotides were obtained from P-L Biochemicals.
Preparation of Blue Sepharose. Blue Sepharose was prepared from Cibacron Blue 3G-A and AH Sepharose 4B according to the procedure of Heyns and DeMoor (5).
Enzyme Assays. Enzymatic activity was measured with a Gilford model 240 recording spectrophotometer. Reaction cuvets with a 1-cm light path contained 120 nmol of PGB_1, 1.08 µmol of NADP, 29 µmol of potassium phospate (pH 7.0), and enzyme in a total volume of 3.0 ml. The reaction was initiated by the addition of enzyme, and the reduction of the pyridine nucleotide was followed by measuring the increase in absorbance at 340 nm, with the cuvet maintained at 25 ± 0.5°. One unit of the enzyme is defined as the amount of enzyme which reduces 1 µmol of NADP per minute when PGB_1 is the substrate. When other cofactors were tested the following amounts were used: NAD, 1.36 µmol; NADH, 680 nmol; NADPH, 540 nmol.
Identification of Products by Thin Layer Chromatography. The reaction mixture contained 60 µg of prostaglandin, 1.36 µmol of NAD or NADH or 1.08 µmol of NADP or NADPH, 10 µmol of potassium phosphate (pH 7.0), and enzyme in a final volume of 1.0 ml. The mixture was incubated for 45 minutes at 37°, the reaction was stopped by adding 1.0 ml of 0.1 M citric acid, and the aqueous solution was extracted with 3 volumes of ethyl acetate. The extract was evaporated to dryness under a stream of nitrogen, redissolved in a small volume of chloroform, and spotted on 5 x 20 cm silica gel thin layer plates. The plates were developed in chloroform: methanol: acetic acid (90:5:5) or benzene:dioxane:acetic acid (30:10:1). The prostaglandins were detected both by ultraviolet light at 254 nm and by exposure to iodine vapors, and they were identified by reference to authentic standards.
Disc Gel Electrophoresis. Disc gel electrophoresis was performed according to the method of Davis (6). Protein bands were stained with Coomassie Blue G-250 (7) and enzymatic activity was located by histochemical staining (3).
Molecular Weight. Estimates of molecular weight were made by gel filtration on Sephadex G-100 or by sodium dodecyl sulfate disc gel electrophoresis (8).
Protein Determinations. Estimates of protein concentration were made by absorption measurements at 280 nm (9), assuming that a solution containing 1 mg/ml of protein has an absorbance of 1.0 in a cuvet with a 1-cm light path.
Enzyme Purification. Three normal human term placentas were chilled on ice immediately after delivery, and all subsequent steps were carried out at 4°. The conditions for homogenization and ammonium sulfate precipitation are identical to those described for the 15-ketoprostaglandin Δ^{13} reductase (4). The 50-80% ammonium sulfate precipitate was collected in a small amount of 5mM potassium phospate (pH 7.0), 20% glycerol, 1mM EDTA (Buffer A) and dialyzed for 36 hours against two 12 L changes of Buffer A. The dialyzed precipitate was centrifuged for 15 minutes at 39,000 x g. The supernatant solution was applied to a DEAE-cellulose column (4 x 35 cm) which was equilibrated with Buffer A. The protein that did not bind to the DEAE column was collected and immediately applied to a Blue Sepharose column

Table 1. Purification of 15-hydroxyprostaglandin (NADP) dehydrogenase

	Step	Activity (Units)	Total Protein (mg)	Specific Activity (mU/mg protein)
1.	Centrifuged homogenate*	-	94,000	-
2.	50-80% ammonium sulfate precipitation	-	-	-
3.	DEAE-cellulose	3.28	25,300	0.130
4.	Blue Sepharose	2.64	80.0	33.0
5.	Hydroxylapatite	1.89	3.16	598

*Material obtained from three term placentas (1120 g of villous tissue).

(2.0 x 8.5 cm) which was equilibrated with 0.01 M potassium phosphate (pH 7.0), 1 mM EDTA. The column was washed with 150 ml of a buffer containing 0.05 M KCl, 0.01 M potassium phosphate (pH 7.0), 1 mM EDTA, and then with 225 ml of a buffer containing 0.1 M KCl, 0.01 mM potassium phosphate (pH 7.0), 1 mM EDTA. The enzyme was eluted by washing the column in a stepwise fashion with 20 ml portions of 0.01 M potassium phosphate (pH 7.0), 1 mM EDTA, containing KCl in 50 mM increments from 0.15 M to 1.0 M KCl. Fractions containing the highest enzymatic activity were combined, desalted, and then applied to a hydroxylapatite column (2 x 5 cm) which was equilibrated with 0.01 M potassium phosphate (pH 7.0). The enzymatic activity did not bind to the column, and those fractions with highest specific activity were combined, concentrated by Diaflo on a UM-10 membrane, dialyzed against a buffer containing 5 mM potassium phosphate (pH 7.0), 1 mM EDTA, 50% glycerol, and stored at -20°.

Preparation of Inhibitory Factor. A placenta was obtained, homogenized, and centrifuged as described under Enzyme Purification, with the exception that the homogenization buffer was 0.01 M potassium phosphate (pH 7.0), 1 mM EDTA. Following centrifugation, the supernatant solution was placed in a boiling water bath and stirred for 1 hour. At the end of that time it was centrifuged at 39,000 x g for 30 minutes. The supernatant solution from this centrifugation was stored at -20°.

RESULTS

The results of the purification are summarized in Table 1. The overall purification was 4,500 fold, and the yield, measured from step 3, was about 60%. The 15-hydroxyprostaglandin (NAD) dehydrogenase and the 15-ketoprostaglandin Δ^{13} reductase were completely removed during purification.

Substrate Specificity. The specificity of the 15-hydroxyprostaglandin (NADP) dehydrogenase for various prostaglandins was determined in both the direction of NADP reduction and the direction of NADPH oxidation.

Table 2. Substrate specificity of purified 15-hydroxyprostaglandin (NADP) dehydrogenase

Prostaglandin	μmol of cofactor utilized/min/ml of enzyme*	products formed
(a) NADP as cofactor		
PGA_1 †	0.548	15-keto PGA_1
PGB_1 †	1.900	15-keto PGB_1
PGE_2	.0027	15-keto PGE_2
$PGF_{2\alpha}$	.0069	15-keto $PGF_{2\alpha}$, PGE_2, 15-keto PGE_2
15-keto PGA_1	not a substrate	----
15-keto PGB_1	not a substrate	----
15-keto PGE_2	not a substrate	----
15-keto $PGF_{2\alpha}$	.0021	15-keto PGE_2
(b) NADPH as cofactor		
PGA_1	not a substrate	----
PGB_1	not a substrate	----
PGE_2	.129	$PGF_{2\alpha}$
$PGF_{2\alpha}$	not a substrate	----
15-keto PGA_1 ‡	1.014	PGA_1
15-keto PGB_1 ‡	3.615	PGB_1
15-keto PGE_2	.210	15-keto $PGF_{2\alpha}$, PGE_2, $PGF_{2\alpha}$
15-keto $PGF_{2\alpha}$	.081	$PGF_{2\alpha}$

*Assay cuvets contained 40 μg of prostaglandin and 1 mg of cofactor in a total of 3 ml of potassium phosphate, pH 7.0. Reaction was started by addition of 9.5 mU of enzyme (5 μl of enzyme from Step 5 of the purification) unless otherwise indicated. The products were identified by thin layer chromatography as described in Materials and Methods.

†3.8 mU of enzyme was used.

‡1.9 mU of enzyme was used.

The rates of the reactions and the products formed are shown in Table 2. Of interest is the small, but significant, reversible conversion of prostaglandins of the E_2 group to their $F_{2\alpha}$ counterparts. Thus this

enzyme preparation also contains 9-ketoprostaglandin reductase (9-hydroxy-dehydrogenase) activity. Assays at various steps of the purification revealed that the relative 9-ketoprostaglandin reductase and 15-hydroxy-prostaglandin dehydrogenase activities remained unchanged during the purification.

Cofactor Specificity. NAD can be utilized in place of NADP, but the rate of conversion of PGB_1 or PGA_1 to the corresponding 15-ketoprostaglandin is about 11 times slower with NAD than with NADP. In the opposite direction both NADH and NADPH can be utilized. The rate of reduction of 15-keto-PGB_1 with NADH is one-fifth the rate with NADPH; however, the rates of reduction of PGE_2, 15-keto-PGE_2 and 15-keto-$PGF_{2\alpha}$ are 50 to 80 times slower with NADH than with NADPH.

Disc Gel Electrophoresis. Disc gel electrophoresis of the purified enzyme preparation revealed two major protein bands plus several minor bands. Histochemical staining showed that the two major protein bands contained 15-hydroxyprostaglandin dehydrogenase activity whether PGA_1 or PGB_1 were used as substrates and NAD or NADP as cofactors. These two bands were estimated by densitometry to comprise approximately 75% of the protein in the sample when stained with Coomassie Blue G-250. A third band containing enzymatic activity was variably noted in several different enzyme preparations.

Placental Inhibitor of 15-Hydroxyprostaglandin(NADP) Dehydrogenase Activity. The 15-hydroxyprostaglandin(NADP) dehydrogenase could not be demonstrated in the centrifuged homogenate when assays were performed spectrophotometrically or by thin layer chromatography because the homogenate contained an inhibitor of the enzyme. Prolonged heating at 100° inactivated the enzyme but did not destroy the inhibitor. The inhibitor is not extractable into organic solvents after acidification to pH 2.0, it is retained by a UM-2 Diaflo membrane, and it is not destroyed by boiling in 0.1N HCl for 1 hour. The elution volume of the inhibitor from Sephadex G-100 suggests that its molecular weight is 6,000 - 7,000. The factor inhibits the oxidation of both PGB_1 and PGA_1 in the presence of either NADP or NAD and the reduction of 15-keto-PGB_1 and PGE_2 in the presence of NADPH. However, concentrations of the factor which are sufficient to cause 100% inhibition of the 15-hydroxyprostaglandin (NADP) dehydrogenase have no effect on the placental 15-hydroxyprostaglandin (NAD) dehydrogenase or the 15-ketoprostaglandin Δ^{13} reductase.

Molecular Weight Estimation. The 15-hydroxyprostaglandin (NADP) dehydrogenase activity is eluted from a Sephadex G-100 column in a single peak corres-

ponding to a molecular weight of 31,000. Disc gel electrophoresis of the fractions from this column revealed that the two bands containing enzymatic activity had not been resolved. These two bands were estimated to have molecular weights of 31,000 and 33,000 by sodium dodecyl sulfate electrophoresis.

DISCUSSION

The human term placenta contains the enzymes necessary for the conversion of prostaglandins of the E and F series to their biologically inactive 13, 14-dihydro-15-keto metabolites (2-4). Since PGA_1 is a good substrate for both the 15-hydroxyprostaglandin (NAD) dehydrogenase (2) and the 15-ketoprostaglandin Δ^{13} reductase[1], it appears that prostaglandins of the A series also can be metabolized along the same pathway in this tissue. Neither the placental 15-hydroxyprotaglandin (NAD) dehydrogenase nor the other 15-hydroxyprostaglandin dehydrogenases that have been isolated (10-12) are capable of oxidizing prostaglandins of the B series, and alternative pathways of metabolism of these compounds have not been identified. The presence in human placenta of a 15-hydroxyprostaglandin dehydrogenase which shows high activity with prostaglandins of the B series, as well as the ability of the placental Δ^{13} reductase to utilize 15-keto-prostaglandins of the B series[1] suggests that placental tissue has the capacity to metabolize the B prostaglandins in the same manner that prostaglandins of the A, E and F series are metabolized.

The physiologic importance of prostaglandins of the A and B series is still unclear. Although these compounds have been isolated from various tissues, recent studies indicate that prostaglandins of the A series are not synthesized in significant amounts *in vivo* but arise from prostaglandins of the E series during isolation procedures (13, 14). Prostaglandins of the B series may be formed similarly (15). Nevertheless, these compounds are active pharmacologically (16, 17). Thus the identification of a route of metabolism of these compounds as well as the existence of an inhibitor may be of eventual therapeutic importance.

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