Purification of prostaglandin E_2-9-oxoreductase from human decidua vera

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Prostaglandin E_2-9-oxoreductase (PGE_2-9-OR), the enzyme which converts prostaglandin E_2 (PGE_2) to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), has been detected in human decidua vera. A 105-fold purification was achieved when the centrifuged homogenate was fractionated sequentially by DEAE–Trisacryl, hydroxyapatite–agarose gel, ultrogel AcA 44 and Matrex gel blue A gel chromatographies. The following kinetic constants for PGE_2-9-OR have been obtained. The equilibrium constant with respect to PGE_2 is 83 μM, the Michaelis constant, K_m, for PGE_2 is 80 μM, for NADPH 1.6 μM. The maximal velocity for the forward reaction is V_1 = .203 pmol/min. The enzyme was inhibited by progesterone, oestradiol-17β, cortisol and pharmaceutical drugs. An activating effect could be demonstrated with Ca^{2+} and oxytocin. The occurrence of PGE_2-9-OR in the decidua vera suggests that this enzyme may be responsible for the transformation of PGE_2 to $PGF_{2\alpha}$ in these tissues. This may be an important mechanism for the initiation and maintenance of uterine contractions.

Prostaglandin E_2-9-oxoreductase *Enzyme purification* *Human decidua vera*

1. INTRODUCTION

Prostaglandin E_2-9-oxoreductase (PGE_2-9-OR), the enzyme system which converts prostaglandin E_2 (PGE_2) to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), has been identified in several tissues [1–7]. PGE_2-9-OR exists in multiple forms [8]. A purification of about 100-fold has been achieved from porcine kidney [8]. The conjugate of PGA_2 and glutathione seems to be a better substrate for PGE_2-9-OR from the kidneys of chicken [9] and rabbits [10] than PGE_2.

The biological importance of that enzyme may be the regulation of the $PGE_2/PGF_{2\alpha}$ ratio in cells. The $PGE_2/PGF_{2\alpha}$ ratio may play a critical role in many biological systems; e.g., contractibility may depend on the availability of sufficient $PGF_{2\alpha}$. PGE_2 and $PGF_{2\alpha}$ have been detected in uterine tissues and the highest concentrations have been found in the decidua vera [11].

Shortly before and during labour in the human there are striking increases in the levels of prostaglandins in amniotic fluid, maternal plasma [12] and uterine decidua vera [13]. Moreover, prostaglandins, when administered to pregnant women at any stage of gestation, induce labour and delivery [14].

The metabolism of prostaglandins in the decidua vera, a tissue that is contiguous with the uterine myometrium, may be of signal importance in the metabolic events that lead to the myometrial contractions of labour in women. The PGE_2-9-OR, which has yet to be defined in human decidua vera tissues, may play a crucial role.

Here we sought to evaluate this enzyme. A method of purification and some properties are described.

2. MATERIALS AND METHODS

2.1. *Enzyme purification*

Human placentae obtained immediately after delivery were chilled on ice. Decidual tissues were

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prepared as in [15] by sharp dissection. Histological examination proved that the decidual tissues were not contaminated with chorion laeve. The tissues from 11 placentae were washed several times with an ice-cold solution of 10 mM phosphate buffer (pH 7.1) containing 1 mM EDTA (sodium salt), 1 mM dithiothreitol and 0.02% sodium azide (buffer A). The tissues were cut into small pieces and homogenized in a Potter Elvehjem homogenizer. The tissue to buffer ratio was 1:4 (w/v). All purification steps were carried out at 0–4°C. The homogenates were centrifuged at 12000 × *g* for 15 min and the resulting supernatant fractions were recentrifuged at 105000 × *g* for 60 min in a Beckman ultracentrifuge model L 5.50. The combined supernatants (fraction 1) were concentrated to 25 ml with an Amicon ultrafiltration system (PM 10 membrane from Amicon, Witten, FRG). Concentrated fraction 1 was applied to a 28 × 1.6 cm column of DEAE–Trisacryl M (LKB, Bromma, Sweden), which had been equilibrated with buffer A. The effluent fractions were pooled (fraction 2) and concentrated immediately to 8.5 ml with the aid of an ultrafiltration cell as described above.

Fraction 2 was applied to a 20 × 1 cm column of hydroxyapatite–agarose gel (LKB) which was equilibrated with buffer A without EDTA, with 20% glycerol (v/v) (buffer B). Enzyme fractions were eluted with the same medium, combined (fraction 3) and concentrated by ultrafiltration to 7.9 ml as described above. The concentrated material from the previous step was applied to a 93 × 2.6 cm column of ultrogel AcA 44 (LKB) equilibrated with buffer B that contained 0.1 M NaCl (buffer C). The eluents (fraction 4) were combined and concentrated by ultrafiltration to 6.0 ml as described above.

Concentrated fraction 4 was applied to a 26 × 1.6 cm column of Matrex gel blue A (Amicon) which had been equilibrated previously with buffer B. The elution was started with 90 ml of the same medium followed by a linear gradient (one buffer chamber contained 90 ml buffer B and the other chamber 90 ml buffer B with 1 M NaCl).

Fractions with the highest specific activity were pooled (fraction 5) and concentrated by ultrafiltration to 5.5 ml as described above. The enzyme was dialyzed against buffer A and stored in aliquots at −18°C.

2.2. *Enzyme activity*

PGE_2-9-OR was measured by a radioimmunological method. The standard assay mixture contained 100 mM Tris–HCl buffer (pH 8.0), 10 μM NADPH, 71 μM PGE_2 and 10–100 μl enzyme solution in a final volume of 1 ml. All assays were conducted with blank controls with denatured enzyme preparations (boiled for 30 min at 95°C). The reaction was allowed to proceed at 37°C for 60 min and terminated by adjusting the pH to 3.5 with 1.5 M citric acid. The samples were processed as in [16].

$PGF_{2\alpha}$ was identified by a highly specific antiserum [17], on TLC plates [18] and by high-performance liquid chromatography [19] (LKB).

For the kinetic studies different substrate concentrations were employed as indicated in section

Table 1

Purification of PGE_2-9-OR from the decidua vera from 4 fresh human term placentae

Fraction	Volume (ml)	Protein (mg)	Activity (pmol/min)	Specific activity (pmol · min^{-1} · mg^{-1})	Enrichment	Yield (%)
105000 × *g* supernatant	94	80.80	289	3.6	–	100.0
Eluate of DEAE–Trisacryl M	69	40.00	211	5.3	1.5	73.0
Eluate of hydroxyapatite–agarose gel	94	11.10	182	16.4	4.6	62.9
Eluate of ultrogel AcA 44	153	4.20	163	38.8	10.8	56.4
Eluate of Matrex gel blue A	42	0.05	19	380.0	105.3	6.6

Throughout purification, enzyme activity was assayed as described in the text by following the production of $PGF_{2\alpha}$

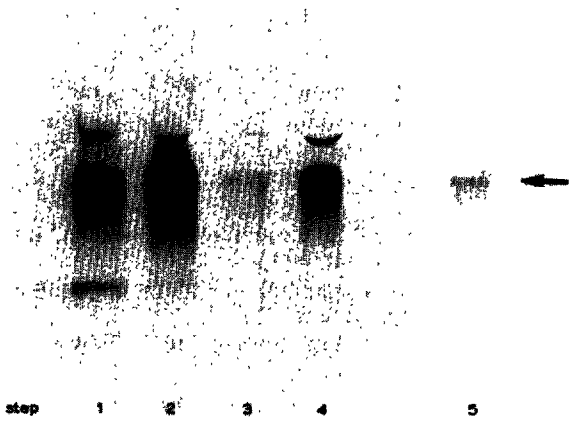

Fig.1. Polyacrylamide gel electrophoresis patterns of prostaglandin E_2-9-oxoreductase. The protein was stained with Coomassie blue. From left to right are shown the individual purification steps (1–5). (←) Prostaglandin E_2-9-oxoreductase.

3. One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 pmol $PGF_{2\alpha}$/min under standard assay conditions. Total protein was determined as in [20].

3. RESULTS

Table 1 illustrates the individual purification steps of the decidual PGE_2-9-OR, which resulted in 105-fold enrichment. Polyacrylamide gel electrophoresis of the last step revealed an almost homogeneous protein fraction (fig.1). An M_r of approx. 33000 could be established by gel filtration. The pH optimum for the forward reaction was 8.3. The kinetic studies were performed with fraction 5. Initial velocities were measured and the enzyme constants calculated as in [21]. Evaluation of the primary and secondary plots provided the constants listed in table 2.

Table 2

Kinetic constants

	Equilibrium constant, K (μM)	Michaelis constant, K_m (μM)	Maximum velocity, V_1 (pmol/min)
PGE_2	83.0	80.5	203.0
NADPH	0.9	1.6	

Material from fraction 5 was used for the kinetic studies. The enzyme constants were calculated as in [21]

Table 3

Effects of various compounds on PGE_2-9-OR activity in human decidua vera tissues

Compound (1 mM)	Remaining activity (%)
None	100
Progesterone	31
Oestradiol-17β	51
Cortisol	45
Vitamin D_3	72
Aspirin	35
Indomethacin	19
Lonazolac	22
Oxytocin	119
Ca^{2+}	115

The enzyme activity was assayed as described in the text by following the production of $PGF_{2\alpha}$

The effects of several biologically occurring compounds and pharmaceutical drugs are listed in table 3. The enzyme was inhibited by progesterone, oestradiol-17β, cortisol, aspirin, indomethacin and lonazolac (Irritren, Byk Gulden, Konstanz, FRG). An activating effect could be demonstrated with Ca^{2+} and oxytocin.

4. DISCUSSION

The enzyme PGE_2-9-OR has been mainly isolated from the kidney [4,8,22]. The activity of the enzyme in human decidua vera tissues has yet to be described. This study demonstrates clearly that PGE_2-9-OR is localized mainly in the cytosolic fractions of decidual tissues.

In view of the physiological and clinical importance of these findings, the ability of the decidua to synthesize $PGF_{2\alpha}$ from PGE_2 suggests that this tissue may play a crucial role in the regulation of labour in women. PGE_2-9-OR prefers NADPH as a cofactor and is therefore independent of the 15-hydroxyprostaglandin dehydrogenase which is present in high concentrations in the human placenta [23].

The isolation of PGE_2-9-OR revealed a 105-fold purification, which is comparable with other reports [8]. Since there might be a specific inhibitor

of PGE_2-9-OR in the placenta [24] the estimation of enzyme activity in crude fractions seems to be unreliable. In the 105000 × *g* supernatant the enzyme activity is readily measured and therefore reflects the starting point. Bisubstrate kinetics give the most reliable values for enzymes that require two substrates. According to [21] 5 different kinetic constants can be obtained.

The K_m values of the substrates were in good agreement with those found in porcine kidney PGE_2-9-OR [8]. The observation that boiled placental fractions seem to contain a specific inhibitor of PGE_2-9-OR suggests that the enzyme function is regulated.

The present preliminary attempts to describe this inhibitor point towards steroids. Equimolar amounts of progesterone, oestradiol-17β and cortisol inhibit PGE_2-9-OR activity by about 50% (table 3). Interestingly, the same was true for the analgetics aspirin, indomethacin and lonazolac to various degrees (table 3). Vice versa, Ca^{2+} and oxytocin seem to stimulate PGE_2-9-OR. These findings could be very important for the regulation of parturition in the human.

In conclusion, this study demonstrates the presence of PGE_2-9-OR in the decidua vera which may be controlled by a variety of compounds. The role of steroids, analgetics and possibly a number of other compounds, remains to be defined in detail.

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