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Human Placental 15-Hydroxyprostaglandin Dehydrogenase

(glycerol stabilization/pyridine nucleotide-linked enzyme/enzyme kinetics)

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ABSTRACT Normal, term, human placentas are a rich source of a 15-hydroxyprostaglandin dehydrogenase. The enzyme is extremely labile, and partial purification could be achieved only after stabilization with glycerol. The instability of the enzyme and its K_m for NAD are indications that it is different from the 15-hydroxyprostaglandin dehydrogenase isolated from swine lung. Human placental tissue should provide a very useful source from which large amounts of highly purified 15-hydroxyprostaglandin dehydrogenase may be obtained.

The first step in the metabolism of the E and F series of prostaglandins is the oxidation of the secondary alcohol group at carbon-atom 15 to a ketone (1, 2). This reaction, catalyzed by a NAD-linked 15-hydroxyprostaglandin dehydrogenase (15-hydroxyprostanoate oxidoreductase, EC 1.1.1.?), yields a product having greatly reduced biological activity (3). Studies of the distribution of activity of the dehydrogenase in swine indicate that it is present in various tissues: kidney, spleen, and lung are its richest sources, while uterus contains relatively little enzyme (4).

During pregnancy, the uterine decidua is an abundant source of prostaglandins (5), but circulating concentrations of these compounds are low until the onset of labor (6). Since it has been suggested that one function of the dehydrogenase is to inactivate circulating prostaglandins (7), the present study was undertaken to determine whether human placental tissue contains this enzyme.

EXPERIMENTAL PROCEDURE

Two spectrophotometric assays were used for measurement of enzymatic activity. Both were performed with a Gilford recording spectrophotometer at 25 ± 0.5°C. The reaction cuvettes (1.0-cm light path) contained, in a volume of 3.0 ml, 100 μmol of potassium phosphate (pH 7.0), 1.35 μmol of NAD, and 114 nmol of prostaglandin E_1 (11α, 15-dihydroxy-9-ketoprost-13-enoic acid), in 0.02 ml of 95% ethanol. Substrate, but not ethanol, was omitted from the blank. The reaction was initiated by the addition of enzyme. The formation of NADH was followed at 340 nm. When the formation of the 15-ketoprostaglandin was measured, 0.3 ml of 1.0 N NaOH was added to each cuvette at an appropriate time, and the development of the chromophore at 500 nm was followed as described by Änggård and Samuelsson (8). Although the first assay was used routinely, both gave similar results. Michaelis constants were obtained from Lineweaver–Burk plots (9).

Purification of the placental dehydrogenase

Normal, term, human placentas were obtained immediately after their delivery and packed in crushed ice until they were homogenized. Within 1 hr of delivery, 75 g portions of villous tissue had been dissected, rinsed with cold tap water, and homogenized in 150 ml of buffer containing 20% glycerol (v/v)–5 mM potassium phosphate–1 mM EDTA (pH 7.0) (medium A). The homogenization was for 1 min at top speed in a Waring blendor. This and all subsequent steps were performed at 0–4°C. The homogenate was centrifuged for 45 min at 10,000 × *g*, the supernatant solution (*Fraction 1*) was decanted, and the residue was discarded. Solid ammonium sulfate was added immediately to the solution to 65% saturation. During this procedure, the pH was maintained near 7.0 by the addition of 3.0 N NH_4OH. After 12–16 hr, the solution was centrifuged for 45 min at 10,000 × *g*. The precipitate (*Fraction 2*) was dissolved in a minimum amount of medium A and was dialyzed for 24 hr against 100 volumes of this buffer. The dialyzed material was centrifuged for 60 min at 20,000 × *g*, and the supernatant solution was applied to a 20 × 200 mm column of DEAE-cellulose (Bio-Rad), which had been equilibrated with medium A. The column was washed with medium A until the absorbance of the eluates at 280 nm fell below 0.3, then elution was started with a linear gradient (one buffer chamber contained 1000 ml of medium A, while the other contained 1000 ml of 20% glycerol–0.7 M potassium phosphate–1 mM EDTA (pH 7.0). 15-ml fractions were collected, and those having the highest specific activity were pooled (*Fraction 3*). This material was then dialyzed overnight against 100 volumes of medium A, applied to a 20 × 150 mm column of hydroxylapatite, and eluted with the same linear gradient that was described above. The fractions having the highest specific activity were pooled (*Fraction 4*). This material was concentrated by ultrafiltration, dialyzed against buffer containing 50% glycerol–5 mM potassium phosphate–1 mM EDTA (pH 7.0), and stored at −20°C. This concentrated enzyme retained full activity for at least 1 month, and was used for most of the studies to be described.

RESULTS AND DISCUSSION

Table 1 illustrates a typical purification of the placental dehydrogenase, which was purified 19-fold, with an overall recovery of 7%.

Because of its extreme lability, the human placental dehydrogenase could not be purified by the procedures described for the swine-lung enzyme (1, 8). Both crude placental homogenates and the purified enzyme (*Fraction 4*) lost more than 90% of their activity when stored at 4°C for 24 hr in 0.1 M potassium phosphate, (pH 7.0). The effects of various agents on the stability of the purified enzyme are illustrated in

TABLE 1. *Purification of 15-hydroxyprostaglandin dehydrogenase from human placenta*

Fraction	Activity (units)*	Total protein (mg)	Specific activity × 10^3 (units/mg protein)
1. Centrifuged† homogenate	13.6	15,750	0.86
2. Ammonium sulfate precipitate	11.2	7,500	1.5
3. DEAE-cellulose	2.1	370	5.7
4. Hydroxylapatite	0.96	60	16.0

* One unit of enzyme is defined as the amount of enzyme that reduces 1 μmol of NAD per min under the conditions stated in the *Experimental* section.

† Material obtained from 280 g of placental tissue.

Table 2. Similar results were obtained with the placental homogenates. Although a solution containing 20% glycerol completely stabilized the enzyme for 24 hr at 4°C, by the end of one week the enzyme had lost 50% of its initial activity. This finding presumably accounts for some of the losses that occurred during the purification of the enzyme. Although glycerol stabilizes another placental enzyme, the 17β-hydroxysteroid dehydrogenase, by preventing it from undergoing cold-inactivation (10), this is not the case for the hydroxyprostaglandin dehydrogenase, since the latter enzyme is more stable at 4°C than at 25°C.

The K_m and V_{max} for various prostaglandins are shown in Table 3. These values closely resemble those obtained for the swine-lung enzyme of comparable purity (11), except that the K_m for prostaglandin E_2 (11α, 15-dihydroxy-9-ketoprosta-5,13-dienoic acid) is smaller than that for prostaglandin E_1 with the placental enzyme and larger with the lung enzyme. The following compounds were not substrates for the placental enzyme: L-lactate, DL-isocitrate, DL-β-hydroxybutyrate, glucose 6-phosphate, testosterone, progesterone, pregnenolone, and cholesterol. The K_m for NAD is 30 μM with prostaglandin E_1 as a substrate. This value is almost 7-fold less than that reported for the swine enzyme (1). NADP is not a cofactor for the enzyme. Michaelis-Menten kinetics were observed for both substrates and cofactor.

TABLE 2. *Protection of dehydrogenase activity by various substances*

Additions	Concentration	Residual activity after 24 hr at 4°C (%)
No additions*		3
2-Mercaptoethanol	14 mM	16
Dithiothreitol	1 mM	55
Glycerol	5% (v/v)	40
Glycerol	10%	60
Glycerol	20%	100

* The storage buffer with no additions contained 0.1 M potassium phosphate–1 mM EDTA (pH 7.0).

TABLE 3. *Substrate specificity of the placental enzyme*

Substrate	K_m (μM)	Relative V_{max} (%)
Prostaglandin E_1	7.7	100
Prostaglandin E_2	5.3	116
Prostaglandin $F_{2\alpha}$ (9α,11α,15-trihydroxyprosta-5,13-dienoic acid)	30.0	78
Prostaglandin A_1 (15-hydroxy-9-ketoprosta-10,13-dienoic acid)	8.7	94

Homogenates of 14 normal, term placentas contained an average of 145 mU/g of tissue ±16 (SE) or 1950 mU/g protein ±259 (SE), when the enzyme activity was assayed at 37°C. Both these values are higher than those obtained for the richest source of this enzyme in the swine (kidney), and are about twice those obtained for swine lung (4). Unfortunately, Änggård *et al.* (4) did not examine swine placental tissue. Although Nakano *et al.* (12) have recently demonstrated that human placental tissue metabolizes prostaglandin E_1, their studies did not measure the 15-hydroxyprostaglandin dehydrogenase content of this tissue. In view of the amount of this enzyme in human placenta, this tissue should be an extremely useful source from which to obtain large amounts of highly purified enzyme.

The high activities of the dehydrogenase in normal placental tissue suggest that this enzyme may function to inactivate prostaglandins formed in the uterine decidua, and that the placenta actually may be a major site of prostaglandin metabolism during pregnancy. Whether changes in the activity of this enzyme are related to the onset of labor remains to be determined.

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