STEREOSPECIFICITY OF HYDROGEN TRANSFER BY THE HUMAN PLACENTAL 15-HYDROXY PROSTAGLANDIN DEHYDROGENASE

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1. Introduction

The human placenta contains a soluble 15-*S*-hydroxy prostaglandin dehydrogenase (EC 1.1.1.141) which is the first enzyme involved in the catabolism of prostaglandins in the placenta [1]. A strict stereospecificity for the 15-*S*-hydroxy group of prostaglandin is a characteristic feature of this enzyme which accepts only NAD^+ as cofactor [2]. The reverse reaction, reduction of the 15-ketoprostaglandin in the presence of NADH is possible in vitro [3]. This property enabled us to study the stereospecificity of hydrogen transfer by the 15 hydroxy prostaglandin dehydrogenase.

When tritium is transferred by a dehydrogenase from a substrate to the carbon atom C_4 of NAD^+, a 4 R or 4 S configuration appears depending on the stereospecificity of the enzyme [4]. Class A dehydrogenases lead to the 4 R configuration, class B dehydrogenases to the 4 S configuration [5]. In the present experiment, 15-keto-PGE_2 was incubated with the enzyme in the presence of 4 S [4-^{3}H]NADH: only the tritium was transferred to the prostaglandin. Thus, our data give evidence that human placental prostaglandin dehydrogenase mediates hydrogen transfer from the 4-pro-S side of NADH to the substrate and should be considered as a B enzyme.

2. Materials and methods

2.1. *Preparation of the enzyme*

The placental cytosol was prepared as previously described [6] and the purification was carried out in potassium phosphate buffer, pH 7.2, 10^{-3} M EDTA, 0.01 M β-mercaptoethanol and 20% glycerol; only the potassium phosphate concentration varied during the following chromatographies:

(1) DEAE-cellulose (Whatman DE 23) (4 × 15 cm), elution with a linear gradient between 300 ml 0.03 M, and 300 ml 0.1 M, phosphate.

(2) Ecteola (Cellex E) (2.5 × 15 cm), elution with a gradient between 0.003 M and 0.06 M phosphate.

(3) Hydroxyapatite (1.5 × 50 cm), elution with a gradient between 0.01 M and 0.06 M phosphate.

(4) G100 Sephadex gel filtration (5 × 120 cm) equilibrated in 0.03 M phosphate.

The elution gradients of the Ecteola and hydroxyapatite chromatographies were established by using an Ultrograd LKB apparatus; the shape of the gradient was checked in order to achieve complete elimination of the estradiol dehydrogenase activity.

2.2. *Incubations*

Enzymatic oxidation of PGE_1 was carried out at 25°C with 0.6 μmol NAD^+, 0.056 μmol [5,6-^{3}H]PGE_1 (2.4×10^5 dpm) (the Radiochemical Centre Ltd, Amersham), 0.5 unit of purified enzyme in 0.1 M phosphate buffer, pH 8.1, to a final volume of 1 ml.

Enzymatic reduction of 15-keto-PGE_2 was performed with 0.6 μmol NADH, 0.06 μmol of 15-keto-PGE_2, 0.5 unit of purified enzyme in 0.1 M phosphate buffer, pH = 7.2, to a final volume of 1 ml.

The reaction products were extracted by ethyl acetate and analysed by thin layer chromatography with chloroform/methanol/acetic acid (90 : 5 : 5, v/v/v) as the irrigant with standard prostaglandins as references. Zones corresponding to the radioactive

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peaks (detected by a Panax radiochromatogram Scanner) were scraped into counting vials containing 15 ml of scintillation mixture (3 g diphenyl oxazole/l toluene) and assayed for radioactivity by liquid scintillation counting.

2.3. *Preparation of 4 S [4-^{3}H]NADH*

NADH labelled with ^{3}H in 4 S position was prepared from [4-^{3}H]NAD^+ (the Radiochemical Centre Amersham; specific radioactivity 2.7×10^7 dpm/μmol) by reduction in the presence of ethanol and liver alcohol dehydrogenase (A enzyme) [7]. The incubation mixture contained 1 ml of ethanol, 1 mg of horse liver alcohol dehydrogenase 4.5 μmol [4-^{3}H]NAD^+ and 0.1 M Tris–HCl buffer pH 9.5 to a final volume of 10 ml. The incubation was run at 25°C; the reaction recorded at 340 nm was terminated by heating at 70°C for 5 min. The remaining [4-^{3}H]NAD^+ was separated from 4 S [4-^{3}H]NADH by DEAE-cellulose chromatography according to [8]. The elution was followed by the absorbance at 260 nm and 340 nm; radioactivity was quantified on 50 μl aliquots of each fraction.

2.4. *Verification of the 4 S [4-^{3}H]NADH and configuration*

Two enzymatic procedures were used:

(1) Reduction of Na pyruvate by 4 S [4-^{3}H]NADH and lactate dehydrogenase (A enzyme). The incubation mixture contained 2.8 μmol of pyruvate, 0.19 μmol of 4 S [4-^{3}H]NADH (4.8×10^6 dpm), 40 μg of lactate dehydrogenase and 0.1 M phosphate buffer, pH = 7.2, in a final volume of 3 ml. A control was run without enzyme. The mixture was incubated for one hour at 25°C. The reduction was stopped by immersion in boiling water for 2 min 0.3 μmol of carrier lactate were added to aliquots of each reaction mixture (0.2 ml), the products were chromatographed on Whatman No. 1 paper and identified as described in [9]. The paper areas corresponding to NAD^+ and lactate were burnt in an Oxymat apparatus and the radioactivity was measured by liquid scintillation counting.

(2) Reduction of oestrone using 4 S [4-^{3}H]NADH and the placental estradiol dehydrogenase (B enzyme). The incubation mixture contained 0.3 μmol of estrone, 0.38 μmol of 4 S [4-^{3}H]NADH (9.6×10^6 dpm), 100 μl of estradiol dehydrogenase (specific activity = 4 U/mg) [10] and 0.1 M Tris–HCl buffer, pH = 7.2, with BSA 3% in a final volume of 3 ml. After a one hour incubation at 25°C, the remaining estrone and the estradiol formed were extracted by chloroform, chromatographed in the chloroform–acetone system (9 : 1, v/v). The estradiol spot was scraped off, extracted and the radioactivity was measured as described in 2.2.

2.5. *Reduction of 15-keto-PGE_2 using 4 S [4-^{3}H] NADH and 15-hydroxy-prostaglandin dehydrogenase*

The incubation mixture contained 0.28 μmol of 15-keto-PGE_2, 0.38 μmol of 4 S [4-^{3}H]NADH (specific radioactivity = 2.55×10^7 dpm/μmol), 1 unit of hydroxyprostaglandin dehydrogenase and 0.1 M Tris–HCl buffer, pH = 7.2, to a final volume of 3 ml. The incubations were run at 25°C. The reaction was followed spectrophotometrically at 340 nm. Controls were run without enzyme. The reaction products were extracted and analysed as described in (2.2.). The radioactivity was measured on aliquots of both the aqueous and the organic phases. The PGE_2 spot localized on the thin-layer chromatography plate after the run was eluted by methanol, and aliquots were counted for radioactivity.

2.6. Oxidation of [15-^{3}H]PGE_2 using NAD^+ and 15-hydroxy-prostaglandin dehydrogenase

The [15-^{3}H]PGE_2 synthetized as described in paragraph 2.5. was diluted with unlabelled PGE_2 and the oxidation reaction was carried out in the conditions described in (2.2.). A reference test was run without enzyme.

3. Results and comments

3.1. *Purity of the 15-hydroxy-prostaglandin dehydrogenase preparation*

The specific activity of the enzyme preparation was 4 U/mg. In spite of this high specific activity, homogeneity was not achieved, since three protein discs were observed on acrylamide gel electrophoresis. However, the purified enzyme was devoid of any enzymatic activity which could interfere with the oxidation of the 4 S [4-^{3}H]NADH used in the hydrogen transfer experiments: PGE_2 was the only product

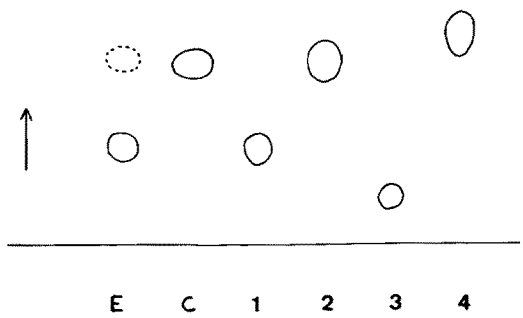

Fig.1. Reduction of 15-keto-PGE_2 by NADH and purified 15-hydroxy-prostaglandin dehydrogenase. After incubation and extraction the products were analysed by thin-layer chromatography as described in paragraph 2.2. E = 15-keto-PGE_2 + NADH + enzyme. C = control without enzyme. Reference products: 1 = PGE_2, 2 = 15-keto-PGE_2, 3 = $PGF_2\alpha$, 4 = 15-keto-13,14-dihydro-PGE_2.

formed when 15-keto-PGE_2 was reduced by the enzyme preparation in the conditions described in (2.2.). The thin-layer chromatography of the extraction products (fig.1) shows that neither products of the 9-ketoreductase nor products of the Δ 13 reductase (NADH dependent enzymes) were detected.

Moreover, when [5,6-^{3}H]PGE_1 was incubated with 15-hydroxy-prostaglandin dehydrogenase and NAD^+, less than 3% of radioactivity was found in the 15-keto-13,14-dihydro-PGE_1 fraction. This radioactivity was certainly due to a contamination by the very close 15-keto-PGE_1 fraction rather than to the actual presence of 15-keto-13,14-dihydro-PGE_1 (table 1). The activity of Δ 13 reductase would have given large amounts of 15-keto-13,14-dihydro-PGE_1 since the oxidation of PGE_1 gave stoichiometric quantities of NADH.

3.2. *Purity of the 4 S [4-^{3}H]NADH*

The enzymatic reduction of [4-^{3}H]NAD^+ in the presence of liver alcohol dehydrogenase gave 4 S [4-^{3}H]NADH with a 75% a yield; after purification the specific radioactivity was 2.55×10^7 dpm/μmol. The configuration of the carbon atom C_4 was checked enzymatically: the coenzyme was used as a cofactor in the reduction of pyruvate by lactico-dehydrogenase and of estrone by estradiol dehydrogenase in conditions described in paragraph 2.4. A 66% oxidation of tritiated NADH (3.45×10^6 dpm) was operated by pyruvate and lactico dehydrogenase with complete retention of tritium on the coenzyme. No radioactivity could be detected in the lactate formed. When estrone was reduced by tritiated NADH (7.4×10^6 dpm) in the presence of estradiol dehydrogenase, 40% of the recovered tritium was incorporated into estradiol while 60% remained in the non oxidized coenzyme; they correspond to the excess of NADH owing to the stoichiometry of the reaction in the conditions used.

Since estradiol dehydrogenase of human placenta was demonstrated to be a B enzyme [11,12], and lacticodehydrogenase to be an A enzyme [13], the configuration of the tritiated NADH that we obtained should be 4 S [4-^{3}H].

3.3. *Stereospecificity of the hydrogen transfer from coenzyme to substrate catalysed by 15-hydroxy-prostaglandin dehydrogenase*

After incubation of 4 S [4-^{3}H]NADH with 15-keto-PGE_2 and the enzyme, approximately 98% of the radioactivity of the organic extract was found to have the same migration as PGE_2 in thin-layer chromatography (table 2). Assuming from the spectrophotometric assay of the [4-^{3}H]NADH that 0.164 μmol of

Table 1
Oxidation of [5,6-^{3}H]PGE_1 by NAD^+ and 15-hydroxy-prostaglandin dehydrogenase

15-Hydroxy-prostaglandin dehydrogenase	[5,6-^{3}H]PGE_1 and NAD^+	Distribution of radioactivity (dpm)			% Radio-activity extracted
		[5,6-^{3}H]PGE_1	15-keto-PGE_1	15-keto-13,14-dihydro PGE_1	
0	+	150 000	0	0	61
+	+	3 900	160 000	4 700	67

After incubation and extraction, the products were separated by thin-layer chromatography and the radioactivity was measured in the respective fractions.

Table 2
Reduction of 15-keto-PGE_2 by 4 S [4-^{3}H]NADH and 15-hydroxy-prostaglandin dehydrogenase

15-Hydroxy-prostaglandin dehydrogenase	15-Keto-PGE_2 and 4 S [4-^{3}H]NADH	Radioactivity after extraction (dpm)		Radioactivity recovered as [15-^{3}H]PGE_2 (dpm)
		Aqueous phase	Organic phase	
0	+	7 $\times 10^6$	0 $\times 10^6$	0
+	+	3.6 $\times 10^6$	4.1 $\times 10^6$	3.9 $\times 10^6$
+	+	3.35 $\times 10^6$	3.93 $\times 10^6$	3.9 $\times 10^6$

[a]Specific activity 2.5 $\times 10^7$ dpm/μmol

Distribution of the radioactivity after extraction and purification by thin-layer chromatography.

the coenzyme were oxidized during the incubation time, according to the stoichiometry of the reaction 4.18 $\times 10^6$ dpm should have been recovered in the 15-^{3}H-PGE_2 extracts. Table 2 shows that the experimental data are in good agreement with these calculations.

These results indicate that the 15-hydroxy-prostaglandin dehydrogenase transfer the [4-^{3}H] of 4 S [4-^{3}H]NADH with a stereospecificity higher than 90% and thus, the 15-^{3}H-PGE_2 obtained should have nearly the same specific radioactivity as the 4 S [4-^{3}H]NADH used for the incubation.

3.4. *Oxidation of the [15-^{3}H]PGE_2 by the NAD^+ and the enzyme*

The [15-^{3}H]PGE_2 obtained in these experiments was oxidized by the 15-hydroxy-prostaglandin dehydrogenase with non radioactive NAD^+ as a cofactor. About 65% of the recovered radioactivity was transferred from [15-^{3}H]PGE_2 to the coenzyme and was detected in the aqueous phase (table 3). The remaining radioactivity was distributed between non oxidized PGE_2 (5%) and another product (30%) which was identified as PGA_2. There is little doubt if any, that this product was formed from PGE_2 during the extraction and thin-layer chromatography steps, since it is well know that PGE_2 easily undergoes dehydration in these conditions [14]. This experiment confirms that the tritium of 4 S [4-^{3}H]NADH is incorporated in position 15 S of PGE_2 by the 15-hydroxy-prostaglandin dehydrogenase.

In conclusion, our data give evidence that the hydride transfer catalyzed by the placental 15-hydroxy-prostaglandin dehydrogenase is direct and that this enzyme should be considered as a B dehydrogenase.

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Table 3
Oxidation of [15-^{3}H]PGE_2 by NAD^+ and 15-hydroxy-prostaglandin dehydrogenase

15-Hydroxy-prostaglandin dehydrogenase	[15-^{3}H]PGE_2 and NAD^+	Distribution of radioactivity (dpm)		
		Aqueous phase	PGE_2	PGA_2
0	+	0	400 000	230 000
+	+	420 000	33 000	200 000

Distribution of the radioactivity after incubation and separation of products by thin-layer chromatography.

References

[1] Jarabak, J. (1972) Proc. Natl. Acad. Sci. USA 69, 533–534.
[2] Thaler-Dao, H., Saintot, M., Baudin, G., Descomps, B. and Crastes de Paulet, A. (1976) in: Advances in Prostaglandins and Thromboxane Research, (Samuelsson, B. and Paoletti, R. eds) Vol. 1, pp. 177–182 Raven Press, New York.
[3] Braithwaite, S. and Jarabak, J. (1975) J. Biol. Chem. 250, 2315–2318.
[4] Cornforth, J. W., Ryback, G., Popjak, G., Donninger, C. and Schroepfer, G. L. (1962) Biochem. Biophys. Res. Commun. 9, 371–375.
[5] Fisher, F., Conn, E. E., Vennesland, B. and Westheimer, F. H. (1953) J. Biol. Chem. 202, 687.
[6] Thaler-Dao, H., Saintot, M., Baudin, G., Descomps, B. and Crastes de Paulet A. (1974) FEBS Lett. 48, 204–208.
[7] Levy, H. R. and Vennesland, B. (1957) J. Biol. Chem. 228, 85–96.
[8] Pastore, E. J. and Friedkin, M. (1961) J. Biol. Chem. 2314–2316.
[9] Zweig, G. and Whitaker, J. R. (1967) in: Paper chromatography and electrophoresis, Vol. 1, pp. 288, Academic Press, New York.
[10] Nicolas, J. C., Pons, M., Descomps, B. and Crastes de Paulet, A. (1972) FEBS Lett. 23, 175–179.
[11] Jarabak, J. and Talalay, P. (1960) J. Biol. Chem. 235, 2147–2151.
[12] Warren, J. C., Gonzalez, M. C. and Soria, J. (1967) Endocrinology 80, 784–788.
[13] Loewus, F. A., Ofner, P., Fisher, H. F., Westheimer, F. H. and Vennesland, B. (1953) J. Biol. Chem. 202, 699.
[14] Hamberg, M. and Samuelsson, B. (1967) Proceedings of the Second Nobel Symposium, (Bergstrom, S. and Samuelsson, B. eds) pp. 63–70, Alquist and Wicksell, Stockholm.