PROSTAGLANDIN ENDOPEROXIDE ANALOGUES AND PROSTAGLANDIN D_2 AS SUBSTRATES OF HUMAN PLACENTAL 15-HYDROXY PROSTAGLANDIN DEHYDROGENASE

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1. Introduction

The current concept for prostaglandin (PG) biosynthesis recently has been enlarged by the discovery of novel transformations of PG endoperoxides which are known as intermediates in the synthesis of the primary PG's E, F_α, and D [1]. Moreover the PG endoperoxides G_2 and H_2 (PGG_2, PGH_2) seem to be more potent than the PGs in platelet aggregation [2], inhibition of adenylate cyclase [3], and contraction of rabbit aorta [4] and human umbilical artery strips [5]. Because of their short half lives in aqueous media (3–5 min) [1], stable endoperoxide analogues were synthesized which are causing comparable effects in some biological systems [6–9].

PGD_2 is biosynthesized by the PG synthetase system [10,11] and has been shown to have important biological activities [4,12,13]. In the platelet aggregation system it even seems to counteract the endoperoxides [2,12].

It should be of interest to know the properties of the endoperoxides and PGD_2 as substrates of 15-hydroxyprostaglandin dehydrogenase (15-HPGDH, EC 1.1.1.141) which is the key enzyme in biological inactivation of most prostaglandins [14]. Recently Sun et al. [15] reported PGH_2 to be substrate of a 15-HPGDH prepared from monkey lung, whereas PGG_2 and PGD_2 were not. Kinetic studies on PGH_2 are not published yet which may be due to its extreme lability. The present investigation has been performed to examine whether two chemically stable PGH_2 analogues and PGD_2 are substrates of a NAD^+-dependent human placental 15-HPGDH and to compare their kinetics with those of PGE_2 and $PGF_{2\alpha}$.

2. Materials and methods

The prostaglandin endoperoxide analogues (15S)-hydroxy-9α,11α-(epoxymethano)prosta-5Z,13E-dienoic acid (U-44069) and (15S)-hydroxy-11α,9α-(epoxymethano)prosta-5Z,13E-dienoic acid (U-46619) as well as PGE_2, $PGF_{2\alpha}$, and PGD_2 were generously supplied by Dr G. L. Bundy and Dr U. Axen, the Upjohn Company, Kalamazoo, Michigan, USA. NAD^+, grade I, was purchased from Boehringer, Mannheim, and silica gel thin layer plates 'DC-Karten SI' from Riedel-de Haën, Seelze-Hannover.

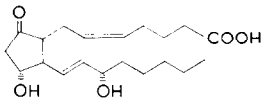
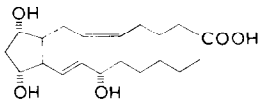
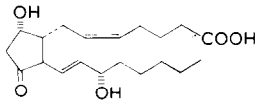
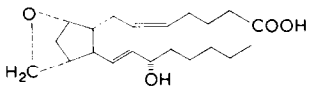
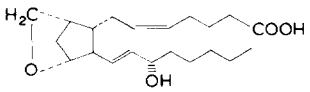
NAD^+-dependent 15-HPGDH was purified from human placenta up to a specific activity of 5200 mIU/mg of protein (25°C, optimal conditions) using a modification of the procedures described by Schlegel and Greep [16] and Jung et al. [17].

Except of PGD_2 complete initial rate patterns were set up using the activity test system described in [17]: initial velocities were determined by measuring NADH formation at 340 nm in 0.1 M triethanolamine buffer (TRAP) at pH 7.4 and 37°C. NAD^+ and $PGF_{2\alpha}$ were varied from 0.025 to 0.5 mM, PGE_2 from 0.0063 to 0.1 mM, and U-44069 and U-46619 between 0.018 and 0.28 mM. The kinetic patterns were evaluated according to Cleland [18].

In order to study PGD_2 as substrate of 15-HPGDH 2 μmol of PGD_2, 2 μmol of NAD^+, and 60 mIU of

enzyme were incubated in 0.1 M TRAP at pH 7.4 and 37°C in a final volume of 2 ml. After 20 min the solution was adjusted to pH 3.5 with 1 n HCl and extracted with ethylacetate. Spectra of the organic phase and its aqueous pH 8 extract were run from 250 to 750 nm in an Unicam SP 1800 double beam spectrophotometer. For thin-layer chromatography the dried ethylacetate extract was evaporated to dryness and redissolved in ethanol. This solution was spotted on heat activated silica gel thin layer plates and chromatographed with benzene/dioxane/acetic acid (20:20:1) for a total length of 14 cm [19]. The plate was dried and developed in ammonia atmosphere or with 10% ethanolic phosphomolybdic acid or cut into slices and eluted with ethylacetate. Initial velocity studies with PGD_2 were performed at pH 8 and 37°C with a fixed NAD^+ concentration of 1 mM recording the formation of a product at 420 nm. PGD_2 was varied from 0.05 to 0.8 mM. All experiments were checked by omitting NAD^+ *or* enzyme from the incubation mixture.

Table 1
Kinetic constants of human placental 15-HPGDH

PG		V_1 (%)	K_m^{PG} (μM)	K_s^{PG} (μM)	K_m^{NAD} (μM)	K_s^{NAD} (μM)	
	PGE_2*	100	3	10	43	130	a)
	$PGF_{2\alpha}$*	98 102	43 45	135 110	44 44	140 110	b) a)
	PGD_2**	1	500				a)
	U-44069*	23 22	21 18	140 80	44 27	160 120	b) a)
	U-46619*	14 15	67 20	250 130	40 23	140 115	b) a)

Kinetic constants which were calculated from sets of initial rate measurements performed at Tübingen are marked with a) at the end of the line. Those from experiments done at Ulm with the same enzyme solution are marked with b). For details see materials and methods.

*These kinetic constants are calculated from complete initial rate patterns at pH 7.4.

**For PGD_2 only apparent constants can be given as it is a very poor substrate. At pH 8 PGD_2 was varied at a fixed NAD concentration of 1 mM.

3. Results

The enzymatic conversion of PGD_2 by 15-HPGDH at pH 7.4 and pH 8 resulted in an increase of absorbance at 340 nm which is mostly due to NADH formation and in the production of a yellow compound with a maximum absorbance at 420 nm which is completely dependent on the presence of PGD_2, NAD^+, *and* enzyme. The yellow colour together with the absorbance at 420 nm disappears by acidifying the solution and reappears at alkaline pH values. This compound was less polar than PGD_2 on thin-layer chromatography. It was detected as a yellow spot in ammonia atmosphere or, after extraction from the plates, by its absorbance at 420 nm. Initial velocity studies at pH 8 – performed by recording the increase of absorbance at 420 nm – resulted in a linear Lineweaver–Burk plot [20]. The apparent Michaelis constant of PGD_2 ($K_m^{PGD_2}$app) was found to be high and the maximum velocity very low compared to the constants of PGE_2 and $PGF_{2\alpha}$ (table 1).

From the complete initial rate patterns of 15-HPGDH forward reaction with the substrates U-44069, U-46619, PGE_2, and $PGF_{2\alpha}$ intersecting double reciprocal primary plots were obtained which were linear within experimental error. The limiting Michaelis constants (K_m), dissociation constants (K_s), and extrapolated maximum velocities (V_1) calculated from the primary plots and the linear intercept and slope replots are summarized in table 1.

4. Discussion

The two PGH_2 analogues U-44069 and U-46619 in which one of the peroxide oxygens of PGH_2 is substituted by a methylene group have a geometry approximating that of PGH_2 [6]. Their effects on platelets, rabbit aorta, and airway resistance are comparable to those of the PG endoperoxides [8,9]. The present study demonstrates that they are substrates of a human placental 15-HPGDH with affinities to the enzyme being in the order of magnitude of $PGF_{2\alpha}$. Their V_1 are considerably lower than those of PGE_2 and $PGF_{2\alpha}$ (table 1). For monkey lung 15-HPGDH PGH_2 itself seems to be a 'better' substrate [15], although a comparison is difficult because of a lack of kinetic studies and a possible conversion of PGH_2 into PGE_2 in the incubation mixture. If our data on the analogues are transferable to PGH_2 and to physiological conditions, it may be possible that PGH_2 – after an eventual release from the PG synthetase – is inactivated via the 15-HPGDH pathway before dramatic effects can be caused.

Compared to PGH_2 the cyclic ethers U-44069 and U-46619 have a considerably improved chemical stability. This is hardly true for their biochemical stability with respect to their metabolism by 15-HPGDH. If they are used for pharmacological studies their conversion into the 15-keto metabolites should be taken into account.

These considerations are not significant in the case of PGD_2 which is no substrate of 15-HPGDH from monkey lung [15] and only a very poor one of the human placental enzyme (table 1). The yellow compound (2_{max} = 420 nm) is product of the dehydrogenase reaction, as it was not formed when NAD^+ *or* enzyme were omitted from the incubation mixture. This is further supported by the linear double reciprocal plot of the kinetic measurements at 420 nm. The colour and absorbance changes of the product between acidic and alkaline solutions may be explained by the pH-dependent formation of an enolate anion of 15-keto PGD_2. Unfortunately we could not further identify this compound, because there was no reference of 15-keto PGD_2 available to us.

The high $K_m^{PGD_2}$app for NAD^+-dependent 15-HPGDH indicates that PGD_2 can not be an inhibitor of the main metabolic pathway of PGE and PGF. Because of the high K_m and low maximum velocity this enzyme can hardly be important in the physiological inactivation of PGD_2. This is in good correlation with the prolonged biological activity of orally or intravenously given PGD_2 as compared with PGE_1 [12]. Therefore another pathway of elimination should exist for PGD_2 or it may occur as part of a pathological development.

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