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Transformation of prostaglandin D₂ to 11-dehydro thromboxane B₂ by Baeyer-Villiger oxidation

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Abstract

Prostaglandin D₂ is one of five chief prostanoids formed in the cyclooxygenase pathway of arachidonic acid oxidation. Except for a single oxygen atom, PGD₂ is structurally identical to 11-dehydro thromboxane B₂ (11d-TxB₂), a urinary metabolite of the pro-aggregatory platelet activator, thromboxane A₂. The close structural relationship suggested that one may be transformed to the other. Accordingly, we tested whether the cyclopentanone of PGD₂ can be expanded to the δ -lactone of 11d-TxB₂ in a Baeyer-Villiger oxidation. Oxidation of PGD₂ with two standard oxidants showed that 11d-TxB₂ was formed only with H₂O₂ but not with peracetic acid. By-products of the H₂O₂-mediated oxidation were hydroperoxide derivatives and isomers of PGD₂. Chemical oxidation of PGD₂ to 11d-TxB₂ may be a model for an equivalent enzymatic transformation, suggesting a possible link in the metabolism of PGD₂ and thromboxane A₂.

Keywords

cyclooxygenase; arachidonic acid; eicosanoid; lactone; peroxyhemiacetal

Introduction

Prostaglandins are formed in the COX-mediated oxygenation of arachidonic acid (1–3). Prostaglandins, together with other eicosanoids formed in the lipoxygenase, cytochrome P450, and autooxidative pathways, are a large and diverse group of lipid mediators (4–6). Additional diversity comes from transformation of related fatty acids, e.g., eicosapentaenoic and docosahexaenoic acids. The resulting products vary in the number and type of oxygen functional groups, cyclizations, and degree of unsaturation (7).

The immediate cyclooxygenase product from arachidonic acid, the prostaglandin endoperoxide PGH₂, undergoes enzymatic transformation to the chief effector prostanoids, PGE₂, PGD₂, PGF_{2 α} , PGI₂, and thromboxane A₂ (3). Prostaglandins are biosynthesized in a tissue-specific manner and act via activation of their cognate G-protein coupled receptors

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Conflict of interest statement

The authors declare that no competing financial or other conflicts of interest exist.

that are involved in the regulation of physiological and pathophysiological cellular events (8).

The premise for this study is based on the close similarity in structure of PGD₂ and 11-dehydro thromboxane B₂ (11d-TxB₂), a urinary metabolite of thromboxane A₂ (9). With identical side chains and hydroxy and oxo functions at the 9- and 11-positions of the prostanoid ring, the only difference is an additional oxygen in 11d-TxB₂, expanding the cyclopentanone of PGD₂ to a δ -lactone. Linear and cyclic ketones can be converted to esters and lactones, respectively, in a Baeyer-Villiger oxidation that inserts a peroxide-derived oxygen next to the carbonyl (10). Thus, we tested feasibility of the Baeyer-Villiger oxidation to transform PGD₂ to 11d-TxB₂ as illustrated in (Fig. 1) (11,12). This resulted in the identification of novel reaction products formed in the transformation.

Materials and Methods

Materials

Prostaglandins were obtained from Cayman Chemical. 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) was obtained from ACROS Organics. Hydrochloric acid, hydrogen peroxide, and triphenylphosphine were obtained from Sigma-Aldrich.

Oxidation reactions

PGD₂ (20 μ g) was dissolved in 20 μ L of HFIP. Water (1.6 μ L), hydrochloric acid (1 N; 0.4 μ L), and hydrogen peroxide (10 M; 0.4 μ L) were added, and the solution was placed at room temperature. After 1 h, the reaction was evaporated to dryness, and the residue dissolved in 20 μ L of methanol. For isolation of products, reactions were scaled 10-fold, and multiple reactions were conducted in parallel.

HPLC

Reactions were analyzed using a Waters Symmetry C18 5 μ m column (4.6 $\times$ 250 mm) eluted with a linear gradient of 20 to 80% acetonitrile in 0.01% aqueous acetic acid over 20 min. The samples were eluted at a flow rate of 1 mL/min, and UV signals at 205 nm were monitored using an Agilent 1200 diode array detector.

LC-MS

Reactions were analyzed using a Thermo TSQ Vantage triple quadrupole MS instrument (Thermo Fisher Scientific) equipped with a heated electrospray interface operated in negative ion mode. Settings for sheath and auxiliary gas pressures, temperature, and interface voltage were optimized using direct infusion of a solution of PGD₂ in acetonitrile/water 1:1 (v/v). The electrospray needle was maintained at 4.0 kV. The ion transfer tube was operated at 300°C. A Zorbax Eclipse Plus C18 1.8- μ m column (2.1 $\times$ 50 mm; Agilent Technologies, Santa Clara, CA, USA) was used for the separation. Water:acetonitrile (80:20, v/v) and acetonitrile:water (20:80, v/v) each containing 0.1% formic acid were the mobile phases (A and B, respectively) used at a flow rate of 0.5 mL/min. A linear gradient was used starting with 100% of solvent A, reaching 100% of solvent B at 5 min, and held for 1 min. The initial conditions were reestablished at 6.01 min and held until 7 min. The signal

intensities obtained for each ion chromatogram are given in exponential format with the maximum intensity set as 100%. Data acquisition and spectral analysis were performed using Thermo Finnigan Xcalibur software.

NMR

NMR Samples were dissolved in 150 μ l of CDCl_3 in a 3 mm-sample tube and analyzed on a Bruker AV-II 600 MHz spectrometer equipped with a cryoprobe. Chemical shifts are reported relative to residual unlabeled solvent ($\delta = 7.25$ ppm).

Results

Oxidation reactions of PGD_2 were carried out in hexafluoroisopropanol (HFIP) in the presence of HCl using peracetic acid or H_2O_2 as oxidant (13). Reaction progress was monitored by RP-HPLC with diode array detection. Use of peracetic acid resulted in disappearance of PGD_2 without detectable formation of 11d-TxB₂. The majority of products obtained with peracetic acid had diene and triene chromophores, likely representing dehydration products (data not shown). When H_2O_2 was used as oxidant 11d-TxB₂ (product **2**) was formed as the intended Baeyer-Villiger product together with several by-products (Fig. 2). Identification of 11d-TxB₂ was accomplished by LC-MS analysis in the MS1 and MS2 modes as well as by co-elution with an authentic standard. The yield of 11d-TxB₂ was 21% based on peak intensities in HPLC analyses, similar in yield to a previous report (11).

LC-MS analyses of crude oxidation reactions (Fig. 3) and isolated peaks were used to determine the molecular weights of the products. The analyses confirmed that 11d-TxB₂ was the main product resulting from insertion of oxygen.

Several by-products of the H_2O_2 -mediated oxidation of PGD_2 were isolated using RP-HPLC. NMR data were collected for some of the products (Table 1 and Supporting Information). Product **1** was tentatively identified as the hydroperoxyhemiacetal of 15-hydroperoxy- PGD_2 (Fig. 4). The structure of **1** was assigned based on LC-MS (m/z 401.0 in negative ion LC-MS; Fig. 3) and from the fact that treatment of **1** with triphenylphosphine (TPP) to reduce peroxide groups yielded PGD_2 . It was unclear whether the hydroperoxy group was located at C-9 or C-15 although comparison to product **4** (see below) suggested the hydroperoxide was more likely located at C-15. The structural assignment is tentative since NMR data for **1** could not be obtained.

Product **3** (m/z 351.0 in negative ion LC-MS; Fig. 3) was identified as an isomer of PGD_2 with the double bond changed from 5*Z* to 5*E* configuration ($J = 15$ Hz; Table 1) (Fig. 4). Peak **4** (m/z 367.1) showed near identical NMR data to PGD_2 with the exception of H-15 that was shifted to 4.36 ppm compared to 4.53 ppm in PGD_2 . Reduction of **4** with TPP yielded PGD_2 , confirming that **4** was 15*S*-hydroperoxy- PGD_2 . NMR data of peak **5** suggested a 13-hydro(pero)xy isomer of PGD_2 with the double bond shifted to 14*E* and side chains on the prostanoic ring in *cis* configuration (12-iso). Treatment of **5** with TPP gave 4 more polar peaks of about equal intensity in LC-MS analyses. Since LC-MS analyses gave inconclusive data for **5** and its reduced products, the structural assignment is considered tentative. None of the products co-eluted with a standard of 15*R*- PGD_2 .

The absolute configuration of products **1-5** as shown in Fig. 4 was determined based on the assumption that the stereochemistry of C-9 was unchanged during the reaction. The $^2J_{\text{H,H}}$ coupling constants used to determine the relative configuration of substituents on the prostanoid ring of **1-5** were obtained through selective spin decoupling experiments (Table 1 and Supporting Information). Reference values for *cis* and *trans* configured hydrogens at the prostanoid ring were obtained from analysis of commercially available prostaglandins with configuration changed relative to PGD₂, namely, 8-iso-PGE₁ and 11 β -PGE₂ (Table 2).

Discussion

Treatment of PGD₂ with H₂O₂ in acidic solvent resulted in Baeyer-Villiger oxidation to 11d-TxB₂. Insertion of oxygen between C-11 and C-12 was compatible with the known mechanism that favors placing oxygen on the side of the higher substituted carbon (14). Thus, Baeyer-Villiger oxidation provides convenient synthetic access to corresponding 11-dehydro thromboxanes starting from the respective PGD precursors. Of two standard peroxide reagents used in Baeyer-Villiger oxidations, only H₂O₂ yielded the intended product with low to moderate yield. Peracetic acid did not form 11d-TxB₂.

Quantification of 11d-TxB₂ in urine is used to assess the anti-platelet effect of low dose aspirin therapy (15,16). The pharmacodynamic target of low-dose aspirin is COX-1 in platelets (17), supplying PGH₂ as substrate for synthesis of thromboxane A₂ (TxA₂) by thromboxane synthase (18). TxA₂ is the major pro-aggregatory eicosanoid in platelets, and its inhibition is the key mechanism for the cardioprotective effects of aspirin (19,20). TxA₂ is hydrolyzed to inactive TxB₂ and further metabolized to 11d-TxB₂ (9,21). Thus, if 11d-TxB₂ may also be derived from PGD₂, application of the former as a biomarker for platelet activation in cardiovascular disease will need to be reconsidered, depending on the efficiency of the metabolic pathway.

Enzymatic Baeyer-Villiger oxidations catalyzed by flavin-dependent monooxygenases are well described in microorganisms, with many of the reactions and enzymes characterized at the molecular level (22,23). In microorganisms the reactions appear to be involved in the mobilization of carbon sources as fuel for cell growth (24) while the mammalian enzymes play a role in the metabolism of xenobiotics (25). Human flavin-dependent monooxygenase 5 (FMO5) has been shown to catalyze Baeyer-Villiger oxidations of two cancer drugs (26,27). Although no endogenous substrates for Baeyer-Villiger oxidations have been described, metabolism of the cancer drugs suggests that the corresponding enzymatic machinery is functional in humans. With regard to transformation of oxylipins Baeyer-Villiger oxidation of a linoleic acid α -ketol, produced by the lipoxygenase-allene oxide synthase pathway in plants (28), was catalyzed by a microorganism isolated from soil (29). The predicted ester product of the Baeyer-Villiger oxidation was unstable and underwent hydrolysis to yield the plant hormone, traumatinic acid (29,30).

Exploring the scope of the Baeyer-Villiger oxidation regarding substrates, products, synthetic application, and its possible occurrence in vivo will add novel facets to the wealth of enzymatic and chemical reactions involved in the formations and metabolism of bioactive lipids.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Proposed conversion of PGD₂ to 11-dehydro thromboxane B₂ (11d-TxB₂) in a Baeyer-Villiger oxidation. The oxygen that expands the cyclopentanone of PGD₂ to the δ-lactone of 11d-TxB₂ is shown in red.

Fig. 2.

Analysis of Baeyer-Villiger oxidation of PGD₂. PGD₂ was reacted with H₂O₂ in acidic hexafluoroisopropanol at room temperature for 1 h. Products were analyzed using RP-HPLC with diode array detection. The chromatogram was recorded at 205 nm.

Fig. 3. LC-MS analysis of a crude Baeyer-Villiger oxidation reaction of PGD₂. The chromatograms represent the ion traces for detection of PGD₂ (*m/z* 351) and products 11d-TxB₂ (**2**; *m/z* 367) and **1** (*m/z* 401).

Fig. 4. Products of the H_2O_2 -mediated oxidation of PGD_2 conducted in the presence of HCl . Products **1** and **5** shown in square brackets have been identified only tentatively.

Table 1.
¹H NMR chemical shifts and correlations for products 3, 4, and 5 formed in the Baeyer-Villiger oxidation of PGD₂.

Spectra were recorded in CDCl₃ using a Bruker AV-II 600 MHz spectrometer equipped with a cryoprobe. Coupling constants of complex signals were determined using selective spin decoupling experiments. NMR spectra are shown in Supporting Information.

Proton No.	3			4			5		
	¹ H (ppm)	Multiplicity	Coupling constant (Hz)	¹ H (ppm)	Multiplicity	Coupling constant (Hz)	¹ H (ppm)	Multiplicity	Coupling constant (Hz)
2	2.34	m		2.41	t	$J_{2,3} = 3.7$	2.37	t	$J_{2,3} = 7.0$
3	1.73	m		1.74	m		1.72	m	
4	2.13	m		2.19	m		2.14	m	
5	5.49	dd	$J_{5,6} = 15.1; J_{5,4} = 8.4$	5.50	dt	$J_{5,6} = 10.5; J_{5,4} = 5.6$	5.43	dt	$J_{5,4} = 7.3; J_{5,6} = 10.4$
6	5.61	dd	$J_{6,5} = 14.9; J_{6,7} = 6.2$	5.58	m		5.34	ddd	$J_{6,5} = 10.8; J_{6,7b} = 6.7$
7a	2.19	dd	$J_{7a,7b} = 14.9; J_{7a,6} = 7.1$	2.30	ddd	$J_{7a,7b} = 10.6; J_{7a,6} = 6.8; J_{7a,8} = 3.8$	2.08	m	
7b	2.48	ddd	$J_{7b,8} = 14.3; J_{7b,8} = 3.9; J_{7b,6} = 8.0$	2.39	m		2.36	m	
8	1.91	ddd	$J_{8,12} = 11.8; J_{7,8} = 3.8; J_{8,9} = 3.6$	2.05	dddd	$J_{8,12} = 15.5; J_{7a,8} = 4.1; J_{7b,8} = 8.1; J_{8,9} = 4.1$	2.08	m	
9	4.54	dt	$J_{9,10} = 2.6; J_{9,8} = 3.6$	4.54	dd	$J_{9,10} = 3.7; J_{9,8} = 3.4$	4.50	dt	$J_{9,10} = 4.8; J_{9,8} = 9.2$
10	2.44	d	$J_{10,9} = 2.8$	2.45	d	$J_{10,9} = 4.2$	2.27	dt	$J_{10a,10b} = 14.5; J_{10,9} = 5.0$
11	-	-		-	-		-	-	
12	2.80	dd	$J_{8,12} = 12.1; J_{12,13} = 8.4$	2.91	dd	$J_{8,12} = 11.9; J_{12,13} = 7.3$	2.85	dd	$J_{8,12} = 6.1; J_{12,13} = 6.1$
13	5.47	dd	$J_{13,14} = 15.4; J_{13,12} = 8.4$	5.70	dd	$J_{13,14} = 15.7; J_{13,12} = 7.3$	4.47	dd	$J_{13,14} = 7.7; J_{12,13} = 7.6$
14	5.62	dd	$J_{4,13} = 15.3; J_{4,15} = 7.3$	5.51	dd	$J_{4,13} = 15.7; J_{4,15} = 8.1$	5.37	dd	$J_{4,13} = 8.3; J_{4,15} = 15.4$
15	4.21	dt	$J_{5,16} = 6.3; J_{4,15} = 6.7$	4.36	dt	$J_{5,16} = 6.9; J_{4,15} = 8.0$	5.87	dt	$J_{5,16} = 6.8; J_{4,15} = 15.4$
16	1.58	m		1.45, 1.61	m		2.06	m	
17, 18, 19	1.23–1.44	m		1.26–1.41	m		1.38, 1.28	m	
20	0.89	t	$J_{9,20} = 6.8$	0.88	t	$J_{9,20} = 7.1$	0.88	t	$J_{9,20} = 7.2$

Table 2.
Coupling constants for *cis* and *trans* configured hydrogens in the prostanoid ring of select prostaglandins.

NMR spectra were recorded in CDCl₃ at 600.13 MHz using a Bruker AV-II 600 MHz spectrometer equipped with a cryoprobe.

Prostanoid
	PGD ₂	8-iso-PGE ₁	11β-PGE ₂
	Coupling constant <i>J</i> (Hz)		
H8-H12	11.6 (<i>trans</i>)	8.2 (<i>cis</i>)	11.9 (<i>trans</i>)
H8-H9	3.5 (<i>cis</i>)	-	-
H11-H12	-	1.6 (<i>trans</i>)	3.4 (<i>cis</i>)