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NMR monitoring of lipase-catalyzed reactions of prostaglandins: preliminary estimation of reaction velocities

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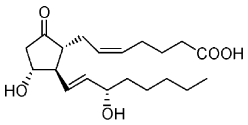
Abstract

The synthesis of methyl esters as well as acetylation of prostaglandins (PG) $F_{2\alpha}$ and E_2 and the corresponding methyl esters catalyzed by *Candida antarctica* lipase B (CALB) has been monitored by using ^{1}H NMR spectroscopy in order to get a quantitative estimation of the velocities of the reactions occurring in the system. The apparent second-order rate constants of acetylation of the 11-OH group of PGs differed by up to two orders of magnitude while the difference between velocities of the CALB-catalyzed methylation of $PGF_{2\alpha}$ and PGE_2 was 2.5 times. The curves describing acetaldehyde formation were determined. The initial rapid consumption of water present on Novozym 435 was followed by an acetaldehyde formation matching the acetylation of prostaglandins.

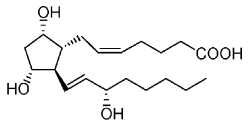
Keywords: Lipase-catalyzed acetylation; Lipase-catalyzed esterification; Low-water media; Reaction monitoring by NMR spectroscopy; Prostaglandin

1. Introduction

The simplest approach to the synthesis of different PGs is based on interconversions starting from PGE_2 (1) [1] or $PGF_{2\alpha}$ (2) [2]. The further development of methodologies for a chemo- and regioselective protection of the functional groups of prostaglandins under mild conditions is of crucial importance. The choice of the methods of synthesis as well as of protecting groups is determined by the stability of target compounds which can often be both acid- and base-sensitive.



PGE_2 **1**



$PGF_{2\alpha}$ **2**

Lipases [3–5] have been shown to catalyze the acylation of E and F type prostanoids with different regioselectivity depending on the origin of the lipase, the structure of PG and the length of the acyl chain [6–9]. Some results have been intriguing. The rates of the acetylation of different but sterically quite similar prostaglandins in organic solvents differ by orders of magnitude. This raises a question about the role of non-steric interactions [10,11] between PG and the lipase in determining different recognition and binding modes of prostanoids by the lipase. Usually the presence of the water in the system has been considered to be essential to maintain the enzyme activity. The hydrolysis of vinyl acetate used as an acyl donor results in the formation of significant amounts of acetic acid and acetaldehyde. This has been described to be the prevailing reaction occurring in the system of the CALB-catalyzed transesterification [12] even under "dry" conditions.

Simultaneous ^{1}H NMR spectroscopic monitoring [12–14] of the progress of acetaldehyde formation in comparison with the other products, monitoring the consumption of the starting materials during the acetylation of a PG and proceeding from the quantities of the starting compounds give a quantitative description of the process useful for scaling up the synthesis. It should be emphasized that ^{1}H NMR spectroscopy is

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a non-invasive, convenient, rapid analytical tool that allows to monitor simultaneously the reactions of molecules of very different by size and properties which is rather problematic to achieve by using other analytical methods.

2. Experimental

2.1. Materials

$PGF_{2\alpha}$ and PGE_2 were purchased from Kevelt Ltd. (Tallinn). The reagents and solvents used were purchased from Merck, Aldrich and Fluka. The preparation of *Candida antarctica* lipase B (Novozym 435; batch LCZ 0001: declared activity approximately 7000 PLU/g and water content 1–2% (w/w)) was a kind gift from Novozymes A/S.

2.2. General methods

^{13}C and ^{1}H NMR spectra were recorded on a Bruker AMX-500 spectrometer. The identification of the acetylation sites was performed by a full assignment of ^{1}H and ^{13}C chemical shifts using ^{1}H–^{1}H and ^{1}H–^{13}C 2D COSY correlation diagrams. The identification of the products was confirmed by comparing NMR spectra with earlier published data [8].

2.3. Lipase-catalyzed acetylation and esterification

The samples of PGs were dissolved in $CDCl_3$ and the solutions were stored on anhydrous Na_2SO_4 for 1 h prior to the use in NMR-monitored reactions. The solution of PG in $CDCl_3$ was introduced into a 5 mm NMR sample tube and vinyl acetate or methanol was added. The proportions of components for individual experiments are given in Table 1. The reaction was started by the addition of Novozym 435. The reactions were performed at room temperature ($20 \pm 2\,^{\circ}C$). The reaction mixture was shaken before each NMR measurement; meantime the sample tube was stored (without agitation) in a nearly horizontal position in order to provide a maximum contact between the solution and the solid catalyst.

2.4. Kinetic experiments

The rates of CALB-catalyzed reactions were measured by using ^{1}H NMR spectroscopy. The reaction progress for the acetylation of $PGF_{2\alpha}$ (Supplementary Fig. 1) and $PGF_{2\alpha}$ methyl ester (Supplementary Fig. 2) was monitored by characteristic signals (Scheme 3) corresponding to the hydrogen atom attached to C_{11}: 2.55 ppm (CHOH) and 3.9 ppm (CHOAc). For the acetylation of PGE_2 (Supplementary Fig. 3) and PGE_2 methyl ester (Supplementary Fig. 4) was monitored by signals of the β-hydrogen atom attached to C_{10}: 2.7 ppm (11-OH) and 2.9 ppm (11-OAc) [15]. Esterification (Supplementary Figs. 5 and 6) was monitored by signals corresponding to the hydrogen atoms of the methoxy group (3.65 ppm). The formation of acetaldehyde was recorded by

Table 1
Investigation of the kinetics of lipase-catalyzed reactions of prostaglandins

Run[a]	PG (mg, μmol)	Novozym 435 (mg)[b]	$CDCl_3$ (ml)	Vinyl acetate (μl, mmol)	CH_3OH (μl, mmol)	Time (min)	Conversion[c] of PG (%)	Formation of acetaldehyde[c] (%)	$k_{II} \pm$ S.E. (ml min^{-1} mg^{-1})[d]
A	$PGF_{2\alpha}$ (24, 67.7)	50	1	60, 0.65		1000	>99	169.4	PG: $(9.2 \pm 0.4) \times 10^{-5}$, Ae: $(20.2 \pm 0.3) \times 10^{-5}$
B	$PGF_{2\alpha}$ Me ester (25, 67.8)	50	1	60, 0.65		2172	84	182.3	PG: $(2.66 \pm 0.2) \times 10^{-5}$, Ae: $(3.16 \pm 0.3) \times 10^{-5}$
C	PGE_2 (24, 68.1)	50	1	60, 0.65		1910	36	131.0	PG: $(0.596 \pm 0.01) \times 10^{-5}$, Ae: $(0.632 \pm 0.02) \times 10^{-5}$
D	PGE_2 Me ester (26, 70.9)	50	1	60, 0.65		2158	10	69.7	PG: $(0.116 \pm 0.03) \times 10^{-5}$, Ae: $(1.88 \pm 0.1) \times 10^{-5}$
E	PGE_2 (6, 17.0)	100	1.25		30, 0.74	800	80		PG: $(2.275 \pm 0.1) \times 10^{-5}$
F	$PGF_{2\alpha}$ (6, 16.9)	100	1.25		30, 0.74	850	95		PG: $(6.375 \pm 0.3) \times 10^{-5}$

[a] Runs A–D are described in Fig. 1, runs E and F in Fig. 3.
[b] Determined with an accuracy of ±1 mg.
[c] 100% corresponds to the starting amount of PG.
[d] The apparent second-order rate constants (per mg/ml of Novozym 435) calculated for the acetylation of the 11-OH group of the prostanoids (PG), for esterification and for the formation of acetaldehyde (Ae).

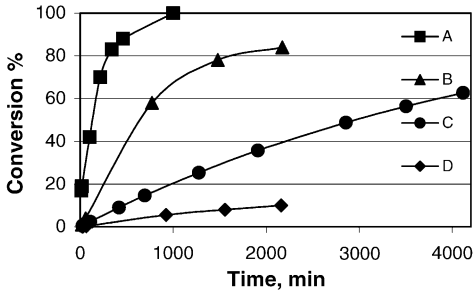


Fig. 1. The reaction progress of the CALB-catalyzed acetylation of the prostaglandins for the runs (see Scheme 3): (A) ($PGF_{2\alpha}$), (B) ($PGF_{2\alpha}$ methyl ester), (C) (PGE_2) and (D) (PGE_2 methyl ester). Lines correspond to the progress curves with estimated rate constants.

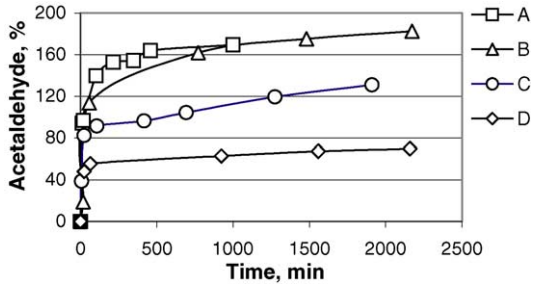


Fig. 2. Acetaldehyde formation during the CALB-catalyzed acetylation of the prostaglandins: the runs A–D (Scheme 3, Fig. 1)—for these runs the reaction conditions were practically identical (Table 1). Hundred percent corresponds to the value equivalent to the total amount of the starting PG.

a signal at 9.77 ppm corresponding to the hydrogen atom attached to the carbonyl carbon atom.

The kinetic curves (Figs. 1–3) were calculated by integrating the above signals followed by standardization against an integrated signal (at 0.85 ppm) of hydrogen atoms of the terminal methyl group of the PG molecule. The latter value corresponds to the total amount of different PGs in the system.

The apparent second-order rate constants (Table 1) for the acetylation, acetaldehyde formation and esterification of prostaglandins were calculated (using least squares method) by fitting the experimental kinetic data to the first-order rate equation. We have presented the apparent second-order rate constants (Table 1, k_{II}) calculated from the pseudo-first-order reaction curves (Figs. 1–3) and total enzyme concentration.

The reproducibility of the results has been confirmed by one additional parallel estimation monitored by NMR as well as by a number of synthetic trials monitored by TLC that did not contradict to the NMR results.

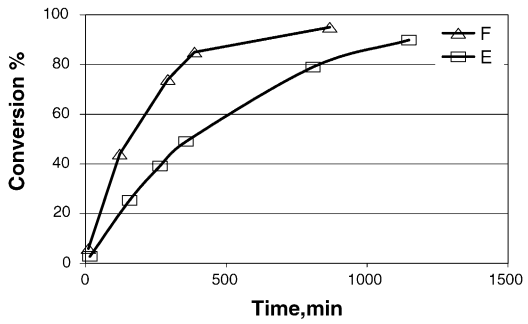


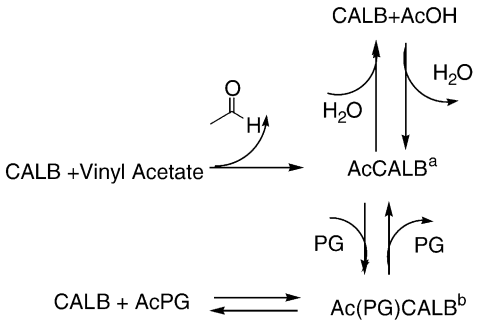
Fig. 3. Kinetics of the CALB-catalyzed esterification of PGE_2 and $PGF_{2\alpha}$; runs E and F (see also Schemes 2 and 3, Table 1). Lines correspond to the progress curves with estimated rate constants.

3. Results and discussion

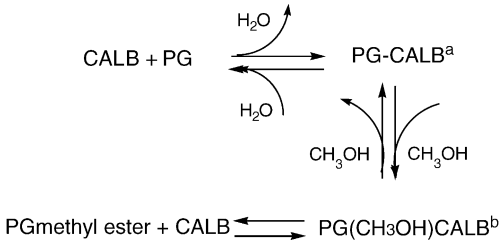
The CALB-catalyzed acetylation of PGE_2, $PGF_{2\alpha}$ and their methyl esters (Schemes 1 and 3, Fig. 1, Table 1) as well as the synthesis of methyl esters of PGE_2 and $PGF_{2\alpha}$ (Schemes 2 and 3, Fig. 3, Table 1) were monitored by using ^1H NMR spectroscopy. The apparent second-order rate constants (k_{II}) for the reactions recorded were calculated (Table 1).

The kinetic schemes of the reactions investigated are presented on Schemes 1 and 2. Vinyl acetate is cleaved by the lipase irreversibly (Scheme 1, step 1). The equilibria of other steps of the process shown in this scheme were shifted to the formation of the end-products by using a large excess of vinyl acetate.

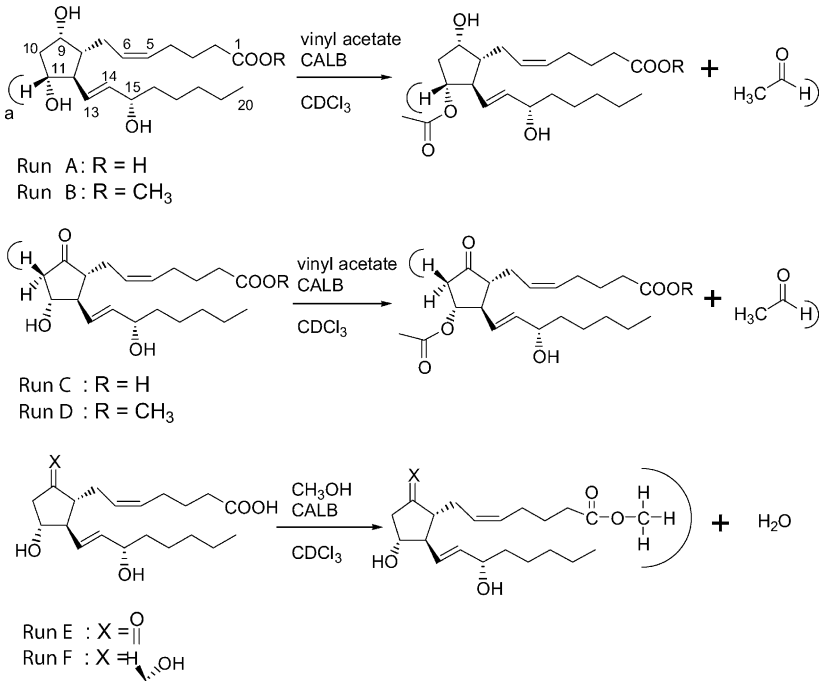
The amount of acetaldehyde formed (Fig. 2, Table 1) corresponds to that of vinyl acetate consumed as well as to the total amount of nucleophiles reacted.



Scheme 1. Kinetic scheme of the CALB-catalyzed acetylation of prostaglandins. Superscript 'a' denotes acetylenzyme; superscript 'b' denotes tetrahedral intermediate.



Scheme 2. Kinetic scheme of the CALB-catalyzed esterification of prostaglandins. Superscript 'a' denotes PG-acylated enzyme; superscript 'b' denotes tetrahedral intermediate.



Scheme 3. Reactions recorded by ^{1}H NMR. Letter 'a' denotes signals corresponding to the hydrogen atoms indicated by the arc lines were used to monitor the reaction progress.

In the reversible PG ester synthesis (Scheme 2) high (≥95%) conversion rates were observed because of the large excess of methanol used.

The velocities of the reactions studied correspond to the two different types and therefore cannot be compared directly because of the different roles of prostaglandins as a nucleophile in acetylation and as a substrate in the CALB-catalytic ester synthesis.

The reactions of PGs monitored by using ^{1}H NMR spectroscopy are depicted in Scheme 3.The differences in rate of acetylation between the 11-and 15-hydroxyl groups of a certain PG as well as in the rate of acetylation of the 11-OH group between different prostaglandins were significant (Table 1).

The acetylation of the 15-OH group of 11-acetyl-PGE_2 was observed (∼5% of conversion during 141 h, run C). This was also detected in the case of 11-acetyl-$PGF_{2\alpha}$ but the rate of formation of the corresponding 11,15-diacetylated PG in the latter case was essentially lower than in the case of PGE_2. The acetylation of the 15-OH group was undetectable in the case of the corresponding methyl esters (runs B and D) during the same period.

The contribution of some functional groups of PGs to the increase of velocity of the acetylation of the 11-OH group of these PGs became evident. The ratios of apparent second-order rate constants were as follows:

(1) The carboxyl group versus methyl ester ∼4/1.
(2) The 9α-hydroxyl group vs. the 9-oxo group ∼20/1.

The formation of PGA_2 (≈5%) was detected in the case of run C (after 141 h).

The kinetic curves of acetaldehyde formation (Fig. 2, Table 1) describe an integrated result of the following processes:

(1) the phase of the rapid consumption of reactive water present on Novozym 435 that completes during 20–25 min;
(2) the acetylation of the 11-OH group of prostaglandins (Fig. 1);
(3) the minute acetylation of the 15-OH group of prostaglandins bearing a free carboxyl group.

A comparison of the kinetic curves describing the formation of acetyl prostaglandins versus respective curves of acetaldehyde formation (Figs 1 and 2, respectively) discloses intriguing features:

(1) Up to 90% of water present in the system (according to the declared for the product value) reacts during the first rapid phase of water consumption.
(2) The part of the kinetic curve of the acetaldehyde formation after the initial rapid water consumption (in the range of 25–2200 min) match the kinetic curves of the acetylation of the PGs (Table 1).
(3) The amount of water reacting during the acetylation of PG seems to depend (by comparison of runs A and D with the others) not only on the amount of water available but also on the structure of the prostanoid acting as a competing nucleophile.

The synthesis of the methyl esters of $PGF_{2\alpha}$ and PGE_2 occurred at the comparable rates.

Associating the carboxyl group of PGE_2 and $PGF_{2\alpha}$ with the imidazole of His224 or with some other basic group in the CALB active site region could undoubtedly be one of the most important interactions determining the mode of coordination of these PGs with CALB during the acetylation. This can be concluded from the reaction rates and regioselectivities of the CALB-catalyzed acetylation. A confirmation to this is provided also by a comparable (4/1) contribution of the carboxyl group versus the ester group to the enhancement of acetylation velocity in both cases.

On the other hand, the 9α-OH group of prostaglandins vs. the 9-oxo group probably contributes to the stabilization of the corresponding tetrahedral intermediates by the formation of additional hydrogen bonds resulting in an increase of the reaction velocity by ca. 20 times for both the pairs of prostaglandins (free acids, methyl esters).

4. Conclusions

(1) Synthesis of methyl esters of PGE_2 and $PGF_{2\alpha}$ occurred at comparable rates showing similar accessibility of the carboxyl group of these prostaglandins to the active site of CALB.
(2) The apparent second-order rate constants of the acetylation of the 11-OH group of prostaglandins investigated differed by up to two orders of magnitude.
(3) The contribution of the carboxyl group of prostaglandins versus the ester group to the acceleration of the acetylation velocity was 4/1. The contribution of the 9α-hydroxyl group vs. the 9-oxo group to the same was determined to be as high as 20/1. Consequently, the carboxyl group as well as the 9α-hydroxyl group participate in the interactions important for the molecular recognition and binding of prostaglandins by acetyl-CALB.
(4) The curves describing acetaldehyde formation were determined. The initial rapid hydrolysis and consumption of reactive water was followed by a process matching the acetylation of prostaglandins.

Acknowledgements

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Appendix A. Supporting material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2004.09.002.

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