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Lipase-catalysed alkoxycarbonylation of thymidine: influence of the carbonate on the regioselectivity of the process

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Abstract

Serine-hydrolases are known to catalyse alkoxycarbonylation processes. In the field of nucleoside chemistry they can be used to perform regioselective transformations at the carbohydrate frame. In this paper we present data regarding the influence of the structure of the carbonate on the regioselectivity of the alkoxycarbonylation of thymidine catalysed by *Candida antarctica* lipase. As expected, assuming the formation of an acyl-enzyme intermediate, the structure of the leaving group portion of the carbonate has no effect, whereas the remaining alcoholic portion of the carbonate has a marked effect.

Keywords: Enzymes; Alkoxycarbonylation; Thymidine; Organic solvents; Regioselectivity; Mechanism

1. Introduction

Enzymes are widely accepted synthetic tools, which can be used to promote selective transformations of substrates [1]. Among them, serine-hydrolases are probably the most commonly employed biocatalysts. Serine-hydrolases catalyse a wide variety of acylation reactions [2] including alkoxycarbonylations [3–9]. The importance of these transformations, especially in protective group chemistry [10], and the selectivity inherent to enzyme-catalysed reactions render these processes extraordinarily interesting. On the basis of these arguments, some stereo-, regio- and chemoselective synthetic routes have been recently described in our group [4–9]. In concrete, this methodology has been successfully applied to develop alternative procedures toward 5′- and 3′-carbonates of nucleosides [4,5,8]. The regioselectivity in these cases was governed by the simple choice of the biocatalyst employed.

Despite the aforementioned interest, to the best of our knowledge, a systematic analysis of the regioselectivity of these transformations as a function of the alkoxycarbonylating agent employed has not yet been published. For that reason we have measured the influence of such factor, in the alkoxycarbonylation of thymidine catalysed by the lipase from *Candida antarctica*. The experimental data obtained are shown in the present work.

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2. Experimental

2.1. Materials

Lipase from *C. antarctica* SP 435L was gently gifted by Novo Nordisk. It was stored under nitrogen at 5°C. Thymidine and other chemicals were purchased from Sigma and Aldrich. THF was dried by reflux over and distillation from sodium. Pyridine was freshly distilled from KOH. Vinyl carbonates **2a–f** were prepared by treating the corresponding alcohol or oxime with vinylchloroformate in pyridine at 0°C. In the same way, acetone oxime carbonates **2g–l** were obtained by treating acetone oxime with the corresponding chloroformate. Products were isolated by conventional procedures, as previously described [4,5].

2.2. Determination of the initial rate of formation of the 5′- and 3′-carbonates of thymidine

A 10 ml solution of dry THF containing thymidine (12.4 mM), carbonate **2a–l** (40 mM) and a small amount of the internal standard (1 mg/ml of pertrimethylsilylated uridine) was prepared in an erlenmeyer flask. The solution was stopped with a rubber septum, introduced in an orbital shaker (250 rpm), and allowed to stand for at least 30 min at 30°C. The immobilised CAL enzyme (50 mg) was then added to the solution, and at regular intervals of time (usually 15–20 min) 200-μl samples were taken from the flask (6–10 samples per reaction). For the reactions with carbonates **2i–l**, samples were taken after several hours, since they proceeded very slowly. Finally, the solvent present in these samples was removed under vacuum. For each carbonate, experiments were done by triplicate.

2.3. Silylation and chromatographic analysis

The silylation procedure employed had been already described by Sweely et al. [11]: 100 μl of pyridine, 8 μl of 1,1,1,3,3,3-hexamethyldisilazane and 4 μl of trimethylsilane chloride were added to each reaction sample. The mixture was stirred and the reaction allowed to reach completion after 5–10 min. At this time, 1 μl was withdrawn from each sample mixture with a Hamilton syringe, and injected into a Hewlett-Packard 5890 Series II gas chromatograph equipped with an HP1 crosslinked methyl silicone gum capillary column. For the detection of the products of reaction with carbonates **2a–i**, isocratic conditions were employed: an oven temperature of 260°C and a head-column pressure of 120 kPa. However, for the rest of reactions this pressure was increased to 140 kPa, and the oven temperature was programmed to be 280°C during 5.5 min, then a temperature ramp of 20°C/min, and finally 7 min at 290°C. Retention times (min) for the 5′/3′-carbonates were as follows: in the reaction with **2a–f** (8.9/9.4), **2g** (8.6/9.2), **2h** (10.2/10.8), **2i** (10.9/11.8), **2j** (8.8/9.2), **2k** (11.4/11.9), **2i** (12.8/14.2). Products were detected with the aid of a flame ionisation detector (350°C), their signal peaks integrated, and their concentrations determined by comparison with the internal standard. In this way, the concentration of the carbonates **3**, **4** could be plotted against the reaction time, and reaction rates were calculated. Reactions never exceeded 10% of total conversion.

3. Results and discussion

One of the most interesting processes developed in our group has been the reaction of alkoxycarbonylation of nucleosides catalysed by enzymes [4,5,8]. Several factors are already known to affect the regioselectivity of this kind of processes, being the most important one the selection of the biocatalyst. Hence, the reacting position can be easily turned from the 5′ to the 3′ hydroxyl group of the nucleoside by simply changing the enzyme. However, it still remained

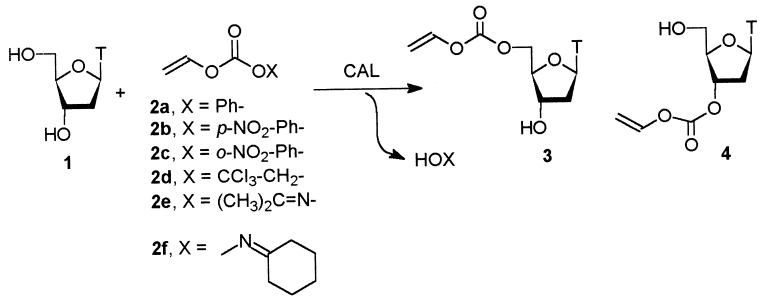

Fig. 1. CAL-catalysed vinyloxycarbonylation of thymidine using carbonates which present different leaving groups.

to be investigated another factor that might have influence on the regioselectivity pattern: the alkoxycarbonylating agent employed.

The alkoxycarbonylation of thymidine catalysed by the lipase from *C. antarctica* lipase (CAL) was the reaction selected to perform such study. Therefore, several carbonates **2a–l** were synthesised and their effect on the regioselectivity of the reaction was measured. The results have been organised in two groups:

(1) Carbonates carrying different leaving group (X), but the same alkoxycarbonylating portion (vinyl carbonates **2a–f**). All of these carbonates give place to the same couple of products (**3** and **4**, Fig. 1). Initial rates for the formation of these two possible vinyl regioisomers were measured, and their ratios (regioselectivity) calculated (Table 1). As we can see, initial rate values were dependent on the leaving group present on the starting carbonate. This indicates that 'acylation' is rate-limiting under these reaction conditions. However, regioselectivity values were independent of the X leaving group. This conclusion was not unexpected since it is probably an indirect evidence of the occurrence of a common intermediate in the reaction mechanism (the vinyloxycarbonyl-enzyme intermediate) when all of these carbonates are employed [12].

It must be pointed out that in the case of the oxime carbonates **2e,f** the regioselectivity values are slightly higher than for the rest of carbonates. Only with these two carbonates the ratio of 5′/3′ product concentration increases as

Table 1
Regioselectivity of CAL-catalysed alkoxycarbonylation of thymidine, as a function of both the leaving group (X) and the alkoxy group (R) present in the starting carbonate[a]

Carbonate	X	$V°(5')$[b]	Regioselectivity[c]	Carbonate	R	Regioselectivity[d]
2a	Ph–	–[e]	–[e]	**2g**	Me–	2.5 ± 5%
2b	*p*-NO_2Ph–	3	1.5 ± 9%	**2h**	^{i}Propenyl–	2.6 ± 8%
2c	*o*-NO_2Ph–	1	1.7 ± 10%	**2i**	Allyl–	1.0 ± 7%
2d	CCl_3CH_2–	1	1.5 ± 9%	**2j**	CCl_3CH_2–	1.7 ± 6%
2e	$(CH_3)_2C$=N–	8	2.1 ± 14%	**2k**	Ph–	20 ± 15%
2f	Cy=N–	7	1.9 ± 11%	**2l**	$PhCH_2$–	4.7 ± 6%

[a] Mean values from experiments performed by triplicate in THF at 30°C.
[b] Initial rate for the formation of the 5′-regioisomer (**3**), relative values: 1 approximately equals 6.6×10^{-7} (M/min) for a CAL concentration of 1 mg/ml.
[c] Regioselectivity = $V°(5')/V°(3')$.
[d] Regioselectivity = [5′]/[3′].
[e] Formation of products was not detected at this temperature.

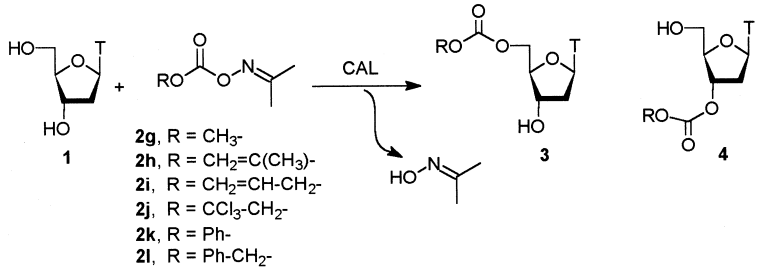

Fig. 2. CAL-catalysed alkoxycarbonylation of thymidine employing different acetone oxime carbonates.

the reaction takes place. [1] This effect is responsible for the higher regioselectivity values obtained with **2e,f** with regard to the rest of vinylcarbonates. In fact, the value for the 5′/3′ concentration relationship extrapolated to time 0 would be closer (around 1.7) to the regioselectivity values obtained with the other carbonates.

(2) Different carbonates carrying the same leaving group (**2g–l**). In this case, different pair of products are formed depending on the carbonate employed (Fig. 2) results have been collected in Table 1. [2] As we can see, regioselectivity is now spread in a wide range of values, going from null regioselection in the case of the reaction with the allyl carbonate **2i**, to the highly regioselective reaction with the phenyl carbonate **2k**. [3] In the last case, the 5′-carbonate of thymidine is formed 20-fold faster than the 3′-carbonate. [4] Obviously, the variation in regioselectivity values comes now from the generation of, and product evolution from different covalent intermediates.

In conclusion, data has been presented in the present work which reflect that the nature of the carbonate employed can have a profound influence on the regioselectivity of the biocatalytic alkoxycarbonylation reaction. However, the influence of the leaving group present in the reacting carbonate has been shown to be practically null.

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[1] One suggestion to explain this observation would be that once the oxime leaving group is released from the carbonate, it behaves as a molecular lubricant, modifying the structural properties of the catalyst, and consequently its regioselectivity.

[2] Initial rates could not be determined with most of carbonates **2g–l**, since reactions proceeded too slowly. For that reason, regioselectivity values were taken to be the mean ratio of 5′/3′ product concentrations at several reaction times.

[3] All thymidine carbonates **3**, **4** were isolated and characterized by ^{1}H and ^{13}C-NMR spectroscopy. Most of them have been already described [4,5,8].

[4] The high regioselectivity of this reaction had been already exploited in a chemoenzymatic route toward 3′-amino-3′-deoxyxylonucleosides [8].