APPLICATION OF PIG LIVER ESTERASE IN THE HYDROLYSIS OF 3,5-DIACETOXYCYCLOPENTENES

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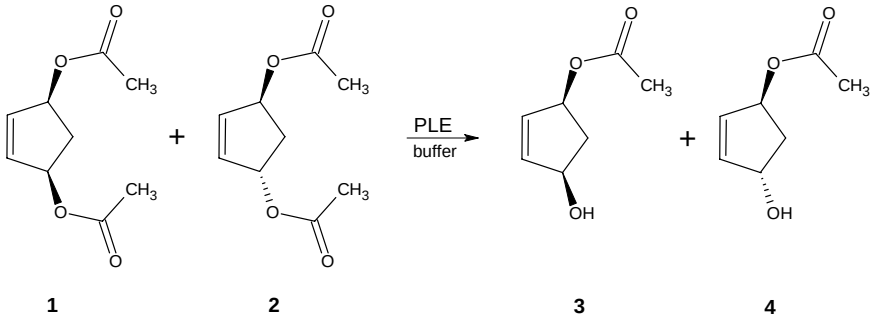
Abstract Enzymatic hydrolysis of a mixture of cis- (**1**) and trans-3,5-diacetoxycyclopentene (**2**) was carried out in the presence of crude and purified pig liver esterase (PLE). Significant differences in reactivity and enantioselectivity of various biocatalyst fractions (obtained in the salting out process and after its immobilization on talc) were observed.

Introduction

Cis-3-acetoxy–5-hydroxycyclopentene (**3**) is an useful starting material for synthesis of prostaglandins and other biologically active compounds. Stereoselective and enantioselective desymmetrisation of *cis*- (**1**) and *trans*-3,5-diacetoxycyclopentene (**2**) mixture is an important step in prostaglandins synthesis. It is well known, that enantioselective hydrolysis of diesters **1** and **2** is catalyzed by lipases: *pig pancreatic lipase, wheat germ lipase*, *Aspergillus niger lipase* and some esterases. There are stereoselective reactions in which isomer *cis* **1** reacted faster [1,2]. The *cis*-3,5-diacetoxycyclopentene hydrolysis (**1**) by *pig liver esterase* (PLE), giving (-)-(5*S*,3*R*)-3-acetoxy-5-hydroxycyclopentene, has been also described [3].

Results

The object of our research was the hydrolysis of the *cis*- and *trans*-3,5-diacetoxycyclopentene (**1** and **2**) mixture catalyzed by PLE (Scheme1). The course of model reaction was monitored by gas chromatography. *Cis*- and *trans*-3-acetoxy-5-hydroxycyclopentene (**3** and **4**) and starting esters **1** and **2** were observed in the reaction mixture.



1 **2** **3** **4**

Scheme 1. The hydrolysis of *cis*- and *trans*-3,5-diacetoxycyclopentene mixture

PLE was obtained from pig liver using a modification of a method reported in literature [4, 5]. It allowed to reduce the research cost. In the presence of crude enzymatic preparation both stereoisomers **1** and **2** were hydrolysed with similar rate. Then the crude enzymatic preparation was purified by ammonium sulphate(VI) outsalting to 60% saturation (P0-60). It turned out, that using this purified enzymatic protein increases the rate of *trans*-3,5-diacetoxycyclopentene (**2**) hydrolysis (Table 1).

Table 1. Course of model hydrolysis catalysed with PLE and P0-60 enzymatic preparations

Enzyme	**Time [hours]**	**Conversion (GC)***	**Isomer *cis* (3)**	**Isomer *trans* (4)**	**Difference in isomers amount**
PLE	26	45,7 %	22,6 %	23,1 %	0,5 %
P0-60	26	49,1 %	19,5 %	29,6 %	10,1 %

*the sum of amount of isomer *cis* (**3**) and *trans* (**4**) in the reaction mixture

The fractionation of crude enzyme PLE was performed by salting out with ammonium sulphate(VI) to 20%, 40%, 60% and 80 % of saturation. In the range of salting to 20% no precipitate has been obtained. Three next enzymatic preparations have been marked as P0-40, P40-60 and P60-80. The first of them (P0-40) was inactive as hydrolysis biocatalyst.
As a result two enzymatic preparations P40-60 and P60-80 were used in the next experiments and the starting enzyme PLE was compared with them.

Table 2. Composition [%] of model reaction mixture containing esters **1**, **2**, **3** and **4**.

	PLE					**P40-60**					**P60-80**				
hours	**1**	**2**	**3**	**4**	**de 4***	**1**	**2**	**3**	**4**	**de 4***	**1**	**2**	**3**	**4**	**de 4***
23	51	38	4	7	27	50	32	7	11	22	56	32	4	8	33
46	46	31	10	13	13	40	15	17	28	24	40	13	19	29	21

*diastereomeric excess of **4**

In Table 2 there are listed compositions of reaction mixtures containing compounds **1**, **2, 3** and **4** after 23 and 46 hours. Under the influence of both purified enzymes P40-60 and P60-80 *trans*-3,5-diacetoxycyclopentene (**2**) reacted faster producing trans-3-acetoxy-5-hydroxycyclopentene excess (**4**) as a result.
The influence of immobilization of all obtained biocatalysts on its activity was checked. The immobilisation of P0-60 enzyme was carried out in sol-gel system, on talc and on the celite. The enzyme reactivity was checked in colour reaction with *p*-nitrophenyl acetate and in model hydrolysis of 3,5-diacetoxycyclopentenes. It was found that immobilization in sol-gel system inactivates PLE. Hydrolysis reactions with enzymes adsorbed on the talc and the celite run much slower (Table 3).

Table 3. The comparison of 3,5-diacetoxycyclopentenes hydrolysis results

Enzymatic preparation	**The protein mass used in reaction (Lowry method)**	**Time [hours]**	**Conversion [%]**
P0-60	376 μg	44	85
P0-60 on celite	Not measured*	44	13
P0-60 on talc	376 μg	44	18
Blind assay	Lack of catalyst	44	2

* Celite impurities interfered with the Lowry assay

After the immobilization on celite the stereoselectivity of hydrolysis of 3,5-diacetoxycyclopentenes decreased. On the other hand, after immobilization on the talc

hydrolysis took place with higher stereoselectivity in comparison with native P0-60 enzyme reaction. (Table 1 and 4.).

Table 4. Comparison of stereoselectivity of the P0-60 immobilized preparations

Enzyme	Conversion (GC)	Isomer *cis* (3)	Isomer *trans* (4)	Difference in isomers amount
P0-60 on celite	48,2 %	20,2 %	28,0%	7.8 %
P0-60 on talk	53,%	20,8 %	32,9 %	12,1 %

In further investigations, enzymes immobilized on talc were used: TPLE, TP40-60 and TP60-80. An enzymatic preparation TP60-80, adsorbed on talc, lost his activity completely. Reactions with enzyme TPLE and TP40-60 run more slowly than previously. The TPLE preparation had higher stereoselectivity, however in the reaction with TP40-60 higher diastereoisomeric excess of *trans*-3-acetoxy-5-hydroxycyclopentene (**4**) was obtained than previously (Table 5).

Table 5. Hydrolysis of 3,5-diacetoxycyclopentenes catalyzed by enzymes immobilized on talc

	TPLE					**TP40-60**				
hours	**1**	**2**	**3**	**4**	**de 4**	**1**	**2**	**3**	**4**	**de 4**
26	51	41	3	4	14	47	27	8	18	39
46	44	31	11	14	12	42	20	28	28	36
68	38	24	16	22	16	38	18	29	29	29

After the reactions were stopped, the mixtures were separated by liquid column chromatography. Enantiomeric ratio of (-) and (+) *cis*-3-acetoxy-5-hydroxycyclopentene (**3**) isolated from all reactions was analyzed by means of HPLC with chiral column (Whelk SS). All results are listed in the Table 6. The obtained *trans*-3-acetoxy-5-hydroxycyclopentene was optically inactive.

Table 6. Enantiomeric ratio of (-) and (+) *cis*-3-acetoxy-5-hydroxycyclopentene (**3**)

Enzyme	***cis*-3-acetoxy-5-hydroxycyclopentene**	
	3(-) / 3(+)	**[α]**
PLE	**34 / 66**	**24,56°**
P40-60	77 / 23	-31,74°
P60-80	77 / 23	
TPLE	**26 / 74**	
TP40-60	73 / 27	

Conclusions

In our research application of *pig liver esterase* to the stereoselective hydrolysis of 3,5-diacetoxycyclopentenes (**1** and **2**) was showed. The obtained preparations: P40-60, P60-80 as well as TPLE and TP40-60 hydrolysed faster stereoisomer *trans* (**2**) that might be

used in enzymatic separation of esters **1** and **2**. Catalytic properties of pig liver esterase could be modified by purification and immobilization in a simple way on talc. The hydrolysis catalyzed by PLE lead to excess of (-)-*cis*-3-acetoxy-5-hydroxycyclopentene, while during hydrolysis with purified preparations P40-60 and P60-80 (+)-*cis*-3-acetoxy-5-hydroxycyclopentene was obtained. Enzyme immobilization has great influence on its catalytic activity.

Experimental part

Preparation of crude pig liver esterase: pieces of fresh pig liver and acetone (300 ml) were homogenized in blender. Precipitate was homogenized again in acetone (150 ml) and then with methylene chloride (150 ml). Obtained precipitate was crumbled and dried under vacuum.

Immobilization of enzymatic preparations on talc (PLE, P40-60, P60-80): Enzymatic preparation (250 mg) was dissolved in phosphate buffer pH 7.00 (5ml). To the solution talc Luzenac was added (1.0 g) and dispersion was shaken in 1.5 hour. Next the mixture was centrifuged. Obtained precipitate was dried under vacuum. Amounts of immobilized proteins were determined by Lowry's method.

*Hydrolysis of 3,5-diacetoxycyclopentene (**1, 2**):* Acetone (2 ml), 3,5-diacetoxycyclopentene (0.5 g; 2.7 mmol) and enzymatic preparation were placed into phosphate buffer pH 7.00 (50 ml) The mixture was stirred at 30°C. Reaction run was monitored by GC. After the completion of reaction, the enzyme was filtered off. Mixture was extracted with diethyl ether. The organic phase was dried and ether was evaporated. Products and substrates were separated by column liquid chromatography (Kieselgel 60, 230-430 mesh, eluent – changing from CH_2Cl_2 to $CHCl_3$).

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