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Journal of Molecular Catalysis B: Enzymatic 26 (2003) 115–118
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Enzymatic selective acylation of glycosides in ionic liquids: significantly enhanced reactivity and regioselectivity

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Received 22 March 2003; received in revised form 14 April 2003; accepted 14 April 2003

Abstract

The enzymatic selective acylations of carbohydrates in ionic liquids were explored in both organic solvents and ionic liquids to see any significant differences in terms of reactivity and regioselectivity between two different classes of reaction media. Monoprotected glycosides (methyl-6-*O*-trityl-glucosides and galactosides) were chosen as the substrates with *Candida rugosa* lipase as an acylation enzyme. Two organic solvents, THF and chloroform, and two ionic liquids, $[BMIM]^+PF_6^-$ ($[BMIM]^+$ = 1-butyl-3-methylimidazolium) and $[MOEMIM]^+PF_6^-$ ($[MOEMIM]^+$ = 1-methoxyethyl-3-methylimidazolium), were employed as reaction media. The enzymatic reactions were performed in the presence of vinyl acetate at room temperature. It was observed that the reactions in ionic liquids took place more rapidly and more selectively than those in conventional organic solvents.

Keywords: Glycosides; Regioselective acylation; Ionic liquid; Enzymatic transesterification; Lipase

1. Introduction

The chemical selective modification of carbohydrates usually requires several protecting and deprotecting steps, since they have multiple hydroxyl groups of comparable chemical reactivity [1]. The enzymatic methods provide a useful alternative to the classical methods for the selective transformations of the polyhydroxyl groups [2]. However, the enzymatic methods often suffer from slow reaction rates and low yields, which are caused by the low solubility of carbohydrates in most conventional organic solvents. Polar solvents such as pyridine, DMSO, DMF, THF may be employed to overcome the solubility problem at the cost of enzyme activity and stability [3]. Recently, a few groups including ours have reported the use of room temperature ionic liquids as useful media for biocatalysis [4]. In some cases, their use enhanced the enzyme activity and selectivity. Ionic liquids are particularly attractive as a new type of alternative media for the enzymatic reactions of carbohydrates because their polarity enhances the solubility of carbohydrates. A recent study has shown that the enzymatic reactions of β-D-glucose proceeded efficiently in ionic liquids with superior regioselectivities to those in conventional organic solvents [4f]. In these reactions, the primary OH group at the C-6 position of substrate was selectively acylated by *Candida antarctica* lipase B in the presence of several secondary OH groups. In this work, we explored the selective

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doi:10.1016/j.molcatb.2003.04.001

acylations of the 6-*O*-protected glycosides with *Candida rugosa* lipase (CRL) in two organic solvents and two ionic liquids to see the effects of using ionic liquids that enhance reaction rate, regioselectivity (toward secondary hydroxy groups) and yield.

2. Experimental

2.1. General

The *C. rugosa* lipase was purchased from Sigma Chemical Co. (St. Louis, MO). Methyl-6-*O*-trityl-D-glycosides were prepared by a conventional method from the methyl-D-glycosides purchased from Sigma. The ionic liquids were prepared according to the literature procedure, [4f,5] and other chemicals were purchased from Aldrich Chemical Co. Chloroform and THF were used after drying according to the conventional methods.

2.2. General procedure for enzymatic acylation

The procedure for the enzymatic acylation of **3b** in ionic liquid **1** is described as a representative. The mixture of **3b** (25 mg, 0.057 mmol), CRL (125 mg, 500 wt.%), vinyl acetate (0.5 ml) in 1 ml of **1** was stirred at room temperature for 5 h. After completion of the reaction, the enzyme was filtered off and the resulting filtrate was extracted with diethyl ether (20 ml × 5) and the combined ethereal phase was concentrated. The residue was subjected to the ^{1}H NMR analysis for the determination of conversion% and regioisomer ratio, and then to column chromatography (silica gel, *n*-hexane/ethyl acetate = 1) to obtain the acetylated product **4b** as the mixture of two regioisomers in 90% yield (24.7 mg, 0.052 mmol).

2.2.1. *Methyl-2-O-acetyl-6-O-trityl*-β-D-*glucopyranoside* (***4a***)

^{1}H NMR (300 MHz, $CDCl_3$, ppm) 7.36–7.40 (m, 6H), 7.16–7.26 (m, 9H), 4.73 (dd, J = 9.3, 7.95 Hz, 1H), 4.27 (d, J = 7.9 Hz, 1H), 3.50–3.55 (m, 2H), 3.44 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$, ppm) 171.58, 144.21, 129.28, 128.63, 127.88, 102.11, 87.76, 76.05, 74.48, 74.34, 73.20, 64.58, 57.19, 21.70.

2.2.2. *Methyl-2-O-acetyl-6-O-trityl*-α-D-*glucopyranoside* (***4b***)

^{1}H NMR (300 MHz, $CDCl_3$, ppm) 7.43–7.48 (m, 6H), 7.34–7.22 (m, 9H), 4.91 (d, J = 3.6 Hz, 1H), 4.72 (dd, J = 10.1 Hz, 3.6 Hz, 1H), 3.93 (dd, J = 9.88 Hz, 8.61 Hz, 1H), 3.64–3.67 (m, 1H), 3.60 (t, J = 8.97, 1H), 3.39–3.41 (m, 2H), 3.38 (s, 3H), 2.15 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$, ppm) 171.64, 144.31, 129.59, 128.59, 127.83, 97.47, 87.72, 73.92, 73.05, 72.14, 70.10, 64.55, 55.74, 21.65.

2.2.3. *Methyl-2-O-acetyl-6-O-trityl*-β-D-*galactopyranoside* (***4c***)

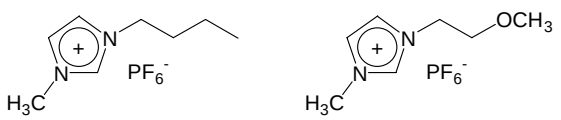
^{1}H NMR (300 MHz, $CDCl_3$, ppm) 7.36–7.40 (m, 6H), 7.16–7.26 (m, 9H), 4.88 (dd, J = 9.6, 8.06 Hz, 1H), 4.121 (d, J = 7.98 Hz, 1H), 3.94 (d, J = 2.19 Hz, 1H), 3.43–3.54 (m, 2H), 3.40 (s, 3H), 3.30–3.38 (m, 2H); ^{13}C NMR (300 MHz, $CDCl_3$, ppm) 172.01, 144.19, 129.27, 128.64, 127.89, 102.38, 87.79, 73.88, 73.55, 70.27, 63.40, 57.71, 21.75.

2.2.4. *Methyl-2-O-acetyl-6-O-trityl*-α-D-*galactopyranoside* (***4d***)

^{1}H NMR (300 MHz, $CDCl_3$, ppm) 7.37–7.39 (m, 6H), 7.16–7.25 (m, 9H), 4.94 (dd, J = 10.1, 3.7 Hz, 1H), 4.83 (d, J = 3.7 Hz, 1H), 3.95 (d, J = 3.02 Hz, 1H), 3.85 (dd, J = 10.1, 3.34 Hz, 1H), 3.76 (t, J = 5.43, 1H), 3.31–3.38 (m, 2H), 3.28 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$, ppm) 172.113, 144.27, 129.27, 128.65, 127.90, 98.06, 87.88, 72.40, 70.99, 69.12, 69.08, 64.04, 55.96, 21.77.

3. Results and discussion

The enzymatic selective acylations of glycosides were examined with *C. rugosa* lipase for four substrates **3a**–**d**. The reactions were carried out at room temperature in the presence of vinyl acetate in four different media; two organic solvents, THF and chloroform, and two ionic liquids, $[BMIM]^+PF_6^-$ (**1**, $[BMIM]^+$ = 1-butyl-3-methylimidazolium) and $[MOEMIM]^+PF_6^-$ (**2**, $[MOEMIM]^+$ = 1-methoxyethyl-3-methylimidazolium) (Fig. 1). The products from each reaction were analyzed by ^{1}H NMR spectroscopy. All the reactions provided 2-*O*-acetyl-glycosides (2-AcG) as the major products together with 3-*O*-acetyl-glycosides (3-AcG) as the



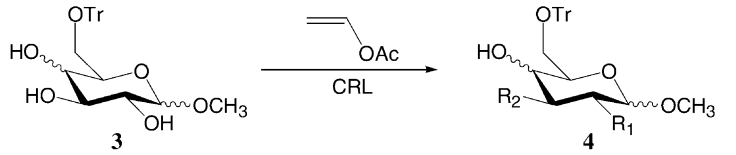
1 2

Fig. 1. Room-temperature ionic liquids.

minor products. The detailed results are described in Table 1.

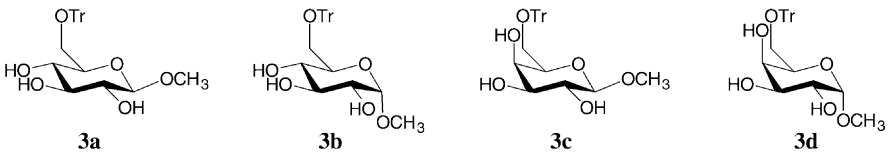
The first important observation from Table 1 is that the reactions carried out in ionic liquids, in general, took place more rapidly and gave higher yields than those in organic solvents. The reactions in THF proceeded slowly and yields were low in most cases. The reactions in chloroform took place relatively more rapidly and afforded higher yields. It is noteworthy that in both chloroform and ionic liquids, the reactions of **3b** and **d** proceeded rather rapidly (3–11 h) while those of **3a** and **c** required rather long reaction times (50–120 h). The second important observation is that all the reactions performed in ionic liquids were significantly more regioselective than in organic solvents. The regioselectivities in two ionic liquids, however, were identical for each substrate. It is noteworthy that the reactions of β-glycosides **3b** and **d** in ionic liquids were highly regioselective to give 2-*O*-acylated

Table 1
CRL-catalyzed acylation of glycosides



3 4

2-AcG: R_1 = OAc, R_2 = OH
3-AcG: R_1 = OH, R_2 = OAc



3a 3b 3c 3d

Entry	Substrate	Medium	Reaction time (h)	2-AcG/3-AcG[a]	Yield (%)[b]
1	**3a**	THF	96	62/38	20
2		$CHCl_3$	90	77/23	25
3		**1**	60	90/10	82
4		**2**	50	90/10	76
5	**3b**	THF	90	80/20	40
6		$CHCl_3$	11	94/6	88
7		**1**	5	>98/1	90
8		**2**	5	>98/1	84
9	**3c**	THF	120	68/32	30
10		$CHCl_3$	90	87/13	82
11		**1**	120	96/4	93
12		**2**	96	96/4	95
13	**3d**	THF	90	94/6	82
14		$CHCl_3$	3	95/5	94
15		**1**	3	>98/1	93
16		**2**	3	>98/1	94

[a] Determined on the basis of the analyses of ^{1}H NMR spectroscopy.
[b] Isolated yields.

products exclusively. The third observation is that the regioselectivity was dependent on organic solvents employed. All the reactions except those of **3d** were more selective in chloroform than in THF. For the reaction of **3d**, almost the same selectivity was observed in both solvents. The regioselectivity was also dependent on the substrates tested: (i) in organic solvents, the acylations of galactosides **3c** and **d** are more selective than those of glucosides **3a** and **b**; and (ii) in both organic solvents and ionic liquids, the acylations of α-glycosides **3b** and **d** are more selective than those of β-glycosides **3a** and **c**.

The first and second observations clearly show the advantages of ionic liquids over organic solvents. The use of ionic liquids enhanced significantly both reaction rate and selectivity to give better yields. The enhancement in reactivity by the use of ionic liquids can be explained partly by the increased solubility of substrates in more polar ionic liquids. The regioselectivity enhancement may be due to more favorable structural adaptation of enzyme in polar ionic liquids. An additional advantage of ionic liquids is that they can be reused readily together with enzyme added. In separate experiments, the reaction of **3d** was repeated twice in ionic liquid **1** with the removal of products by simple extraction between runs (3 h each run). Both the first and second runs gave 2-*O*-acylated products exclusively in good yields (92% and 89% isolated yields, respectively).

4. Conclusion

This work has demonstrated that the enzymatic reactions of carbohydrates in ionic liquids proceeded more rapidly and selectively and provided higher yields than those in organic solvents. The results indicate that ionic liquids are particularly useful as the media for enzymatic transformations of polar substrates that are difficult to dissolve in conventional organic solvents. Accordingly, the scope of enzymatic transformations in ionic liquids can be expanded to a wide range of polar compounds including polysaccharides and nucleotides [6].

Acknowledgements

This work was supported by the Korean Ministries of Science/Technology (the NRL program, 2000–2003) and Education (the BK21 program, 2002–2003), and POSCO (2001–2003).

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