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Influence of ionic liquids on the rates and regioselectivity of lipase-mediated biotransformations on 3,4,6-tri-*O*-acetyl-D-glucal

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Abstract

Lipase-mediated regioselective biotransformations such as hydrolysis and alcoholysis of 3,4,6-tri-*O*-acetyl-D-glucal, **1** have been studied in organic solvent, tetrahydrofuran (THF) and two different ionic liquids, namely 1-butyl-3-methylimidazolium hexafluorophosphate, [bmim]PF_6 and 1-butyl-3-methylimidazolium tetrafluoroborate, [bmim]BF_4. The influence of different reaction media on the rates and regioselectivity of enzyme catalysis has been demonstrated. A marked regioselectivity towards the formation of 4,6-di-*O*-acetyl-D-glucal, **2** was observed in [bmim]PF_6 with 84% product formation after 6 h with 98% selectivity in hydrolysis and 48% after 8 h with 98% selectivity in alcoholysis.

Keywords: 3,4,6-Tri-*O*-acetyl-D-glucal; Lipase; Regioselectivity; Hydrolysis; Alcoholysis; Ionic liquids

1. Introduction

Despite the increasing speculations about the environmentally benign nature of some ionic liquids, broadly they continue to be the solvents of choice for various reactions. This is evident from the ongoing incessant exploration to tap their potential and make the most out of them, not only in chemical processes but also in the enzyme-catalysed transformations. Their versatility as solvents for such diverse applications is due to the favourable attributes of an 'ideal solvent' possessed by them, which are now not new to the chemists [1,2]. Interestingly, in the arena of enzyme-catalysed reactions, these novel reaction media have opened up new prospects [3]. The compatibility of lipases with the ionic liquids has made it possible to resolve kinetically, the commercially important substrates with poor or practically no solubility in the conventional organic solvents, by exploiting their diverse solvating ability. Besides, this medium is not only known to have positive influences on the enzyme-stability [4,5] and activity, [6,7] but also on the enantioselectivity [8–10] of the reactions catalysed by them [11]. The recent studies have revealed that even the regioselectivity of the enzyme catalysis in acylation of sugars is significantly enhanced in the ionic liquids, when compared with conventional organic solvents [12,13].

We earlier investigated the transesterification reaction catalysed by the lipases focusing on the initial rate of reaction in ionic liquids [7]. We attempted to decipher the influence of ionic liquids on the catalytic activity of lipase and also compared them with the conventional organic solvents as the reaction media. Furthermore, the ionic liquid was also demonstrated as a medium for the lipase-catalysed aliphatic polyester synthesis [14]. In continuation of our investigation to envisage the influence of reaction media on several attributes of enzyme catalysis, we herein report the regioselective biotransformations on 3,4,6-tri-*O*-acetyl-D-glucal.

The glycals, 1,2-unsaturated derivatives of pentoses and hexoses are among the most versatile chiral building blocks. Glucal and related compounds belonging to a broad category

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of glycals, have been the subject of considerable interest in carbohydrate chemistry [15], oligosaccharide synthesis [16,17] and in the development of combinatorial synthesis of oligosaccharide libraries [18]. Such compounds have shown a tremendous utility as precursors of *C*-glycosides [19,20] and rare sugars [21]. Thus, various regioselectively acylated analogues of such compounds are warranted for the synthetic manipulations. The chemical methods leading to such analogues involve multi-step protection and deprotection procedures. In contrast, the worth of enzymes as proficient catalyst for a multitude of the stereospecific and regioselective reactions imperative for carbohydrate synthesis is well recognised. The high degree of regioselectivity exhibited by the enzymes in certain sugar molecules is especially interesting, as this fine distinction between two or more secondary −OH groups would be difficult to accomplish chemically. Such reactions have been extensively investigated [22–26]. In 1989, Holla reported the regioselective biotransformations on glycals using a variety of enzymes. High yields of fully hydroxyl differentiated glucals and galactals were prepared via lipase-catalysed hydrolysis and acetylation reactions [27]. Followed by that, in 1994 Waldmann and Heuser [28] reported acetylesterase-catalysed regioselective deprotection of acetylated glucal. Sugai et al. [29] elegantly utilised the regioselectivity of lipase PS to achieve selective acylation on the 6-position of *p*-methoxyphenyl 2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside (and related substrates).

We attempted *Pseudomonas cepacia* lipase (supported on celite), PS-C-mediated hydrolysis of 3,4,6-tri-*O*-acetyl-D-glucal, **1** in two different ionic liquids, viz. 1-butyl-3-methylimidazolium hexafluorophosphate, [bmim]PF_6 and 1-butyl-3-methylimidazolium tetrafluoroborate, [bmim]BF_4 and in tetrahydrofuran (THF), since it is a preferred water miscible organic solvent employed for biotransformations. In a non-aqueous approach, we attempted the lipase-mediated alcoholysis of **1** in the above-mentioned media. The regioselectivity towards the formation of 4,6-di-*O*-acetyl-D-glucal, **2** was observed in both the approaches. The studies revealed that the ionic liquids influence the rates and regioselectivity of lipase-catalysed biotransformations. The ionic liquid, [bmim]PF_6 gave maximum product formation, i.e. 84% after 6 h with 98% selectivity in hydrolysis and 48% after 8 h with 98% selectivity in alcoholysis protocol.

2. Experimental

2.1. Materials

3,4,6-Tri-*O*-acetyl-D-glucal was prepared by the method described earlier [30]. Lipase PS-C was received as gift from Amano Pharmaceuticals, Japan. The ionic liquids [bmim]PF_6 [31] and [bmim]BF_4 [32] were prepared by methods reported earlier.

2.2. General experimental procedure for hydrolysis and alcoholysis

In a typical experimental procedure, to the solvent (THF or [bmim]PF_6 or [bmim]BF_4, 1 ml), **1** (0.367 mmol), lipase PS-C (15 mg, 30 U/mg) and phosphate buffer (NaH_2PO_4/Na_2HPO_4) of pH 7 (0.1 M, 1 ml) were added in the hydrolysis experiments. In alcoholysis, decanol (1.101 mmol) was added instead of phosphate buffer. The reaction mixture was stirred at room temperature for fixed intervals of time (2, 4, 6 and 8 h). The volatile organic solvent (if any) was evaporated under reduced pressure and the products were extracted using diethyl ether (3×15 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The resultant syrup was diluted and assayed on HPTLC after adding isopropyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside, **3** as an external standard. In the preparative experiments, the reactions were carried out on a 2 mmol scale and the work up was same as described above. The product **2** was purified by the silica gel column chromatography and characterised by IR, ^1H and ^{13}C NMR spectroscopy.

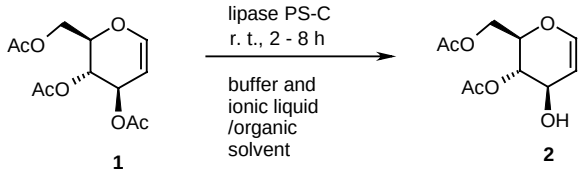
2.3. High-performance thin-layer chromatography (HPTLC)

The percentages of **1** and **2** at fixed time intervals during the reactions, in different solvents, were monitored by HPTLC, and computed from the respective calibration curves. The samples (standards for calibration curve and extracts from reaction mixture) were assayed employing the CAMAG Linomat IV sample applicator (5 μl sample applied for each analysis) on silica gel 60 F_{254} pre-coated plates. The plates were developed in a twin trough chamber using petroleum ether:ethyl acetate (7/3, v/v) mobile phase and derivatised by dipping in derivatising agent (1% anisaldehyde, w/v, 1% concentrated H_2SO_4, w/v, and 1% glacial acetic acid, w/v, in methanol) and subsequently heating in an oven at 100 °C for 10 min. The plates were scanned on a CAMAG TLC densitometric scanner II (tuned at 550 nm) equipped with the Cats 3.0 version software to obtain the chromatograms. The calibration curves for the **1** and **2** were obtained in the concentration range of 4.595–18.38 μmol ml^{-1}. The preparation of the respective solutions in this concentration range was achieved by dissolving 0.0919, 0.1838, 0.2757 and 0.3676 mmol of **1**/**2** in CH_2Cl_2, such that all the solutions were 0.5% (w/v) with respect to the external standard **3**. The net volume of the solution was 20 ml.

3. Results and discussions

3.1. Regioselective hydrolysis

In a preliminary experiment, we employed THF/0.1 M phosphate buffer of pH 7.0 used 1/1 by volume as a medium



Scheme 1. Regioselective hydrolysis of 3,4,6-tri-*O*-acetyl-D-glucal using *Pseudomonas cepacia* lipase.

for hydrolysis of **1**. The reaction was stirred at room temperature and was monitored at regular intervals of time for 8 h. The reaction fetched **2** as a major product. However, the careful examination of the reaction mixture on TLC revealed the presence of other di- and tri-deacetylated products from **1**. In contrast to this, the reaction in [bmim]BF_4 appeared relatively clean, but the extent of conversion appeared to be low even after 8 h of reaction time. We then attempted the same reaction in water immiscible ionic liquid, [bmim]PF_6 with aqueous buffer. Recently, the strategy of executing reactions in biphasic reaction media is being increasingly exploited owing to the merits associated with such systems [33]. The reaction in [bmim]PF_6 was clean, i.e. completely devoid of other products in the initial stages. These differences in the product distribution during the regioselective transformation prompted us to systematically investigate the influence of different reaction media on the regioselectivity of the reaction. We planned a study wherein the hydrolysis was attempted in all the above mentioned solvents (Scheme 1). The reactions were arrested at regular time intervals and the reaction mixtures were assayed by HPTLC. The HPTLC analysis not only facilitated the examination and comparison of the initial rates of formation of **2** at different time intervals, but also gave a quantitative picture of selectivity towards **2** in these reaction media during the course of reaction. High initial rate of regioselective deacetylation was observed in [bmim]PF_6 as a solvent giving 75% formation of **2** after 2 h. The extent of product in THF was found to be 45% of that obtained in [bmim]PF_6 after 2 h, whereas in [bmim]BF_4, the number was far from being impressive as illustrated in Fig. 1. Subsequently, when the reaction time was prolonged, the extent of product formed increased until it reached the maximum of 84% in [bmim]PF_6 after 6 h and then remained constant. In THF, the maximum extent of product was limited to 60% after 8 h whereas in [bmim]BF_4, the yields were too less, i.e. 17% after 6 h and did not increase beyond that. On the contrary, in [bmim]BF_4 the extent of **2** decreased after 6 h due to its subsequent deacetylation and was found to be only 13% after 8 h.

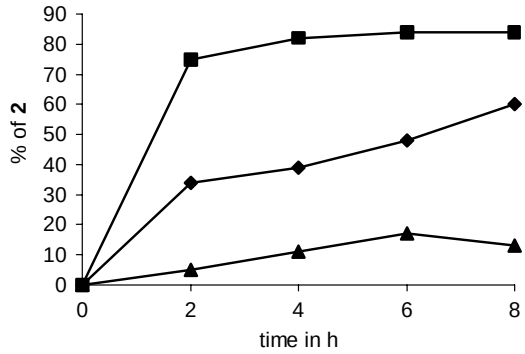
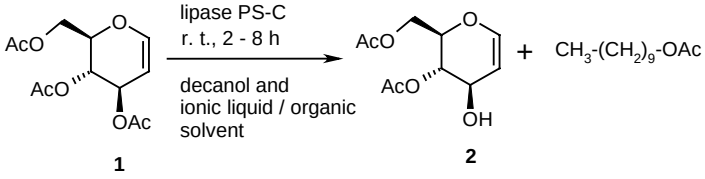


Fig. 1. Regioselective hydrolysis of 3,4,6-tri-*O*-acetyl-D-glucal catalysed by lipase PS-C in different reaction media, viz. tetrahydrofuran (◆), [bmim]PF_6 (■) and [bmim]BF_4 (▲).

The regioselectivity of the reaction was quantified in terms of the percentage selectivity, i.e. the ratio of percentage of **2** formed to the percentage of **1** consumed. We computed the percentage selectivities towards **2** in these media at the time of maximum product formation, to adjudge the preparative value of the protocol. The selectivity was found to be maximum in [bmim]PF_6, i.e. 98% after 6 h. In THF, it was 62% after 8 h and in [bmim]BF_4, the selectivity was 68% after 6 h. Moreover in [bmim]BF_4, the percentage selectivity falls off to 59% after 8 h.

3.2. Regioselective alcoholysis

In a non-aqueous approach to the target **2**, we attempted lipase PS-C-catalysed alcoholysis of **1** using a fatty alcohol, decanol in ionic liquids and organic solvent. Similarly, the alcoholysis was systematically investigated using HPTLC in THF, [bmim]PF_6 and [bmim]BF_4 as the reaction media for the period of 8 h (Scheme 2). The results revealed that the extent of formation of **2** in THF was found to be relatively greater than that observed in the two ionic liquids. In THF, after 2 h, 36% of **2** was obtained and the extent increased thereafter to 55% after 8 h. Among the two ionic liquids investigated for alcoholysis, [bmim]PF_6 proved to be relatively better medium, both in terms of rates and extent of product formation. In [bmim]PF_6, the extent of **2** was 48% after 8 h, which is marginally less than that observed in THF. The extent of product formation was relatively poor in [bmim]BF_4, i.e. 13% after 2 h and in the later stages of the



Scheme 2. Regioselective alcoholysis of 3,4,6-tri-*O*-acetyl-D-glucal with decanol employing *Pseudomonas cepacia* lipase.

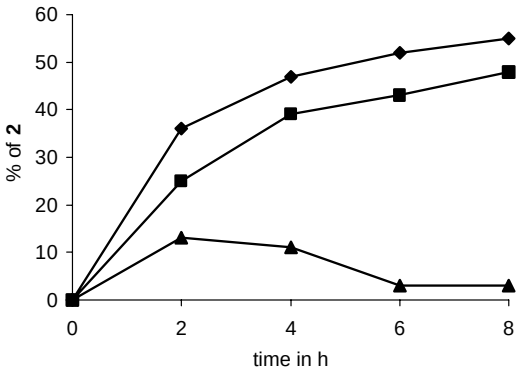


Fig. 2. Regioselective alcoholysis of 3,4,6-tri-*O*-acetyl-D-glucal catalysed by lipase PS-C in different reaction media, viz. tetrahydrofuran (◆), [bmim]PF_6 (■) and [bmim]BF_4 (▲).

reaction, the extent of **2** further decreased as illustrated in Fig. 2.

In alcoholysis, the percentage selectivity was found to be 98% in THF after 6 h. Thus, strategically alcoholysis in THF exhibited better selectivity than the hydrolysis. The fact is even evident from the formation of di- and tri-deacetylated products observed in the HPTLC chromatograms of the reaction mixtures of hydrolytic protocol in THF. However, in THF, the overall product formation was marginally higher in hydrolytic protocol. In alcoholysis, the best selectivity was observed in [bmim]PF_6, i.e. 100% after 6 h and at the time of maximum product formation, it was 98% after 8 h and poor selectivity was observed in [bmim]BF_4, i.e. 22% after 2 h.

3.3. The preparative experiments

The preparative experiments were conducted under the conditions (solvent and time) at which the maximum extent of product formation was indicated by the HPTLC analysis. The reactions were carried out on a 2 mmol scale with respect to the substrate. As can be seen from the analysis presented above, it is evident that THF and [bmim]PF_6 can act as better solvent candidates for trying the preparative experiments. We thus conducted the preparative experiments for obtaining the target in both the solvents via both hydrolysis and alcoholysis at the optimal times of the respective reactions. The reactions were quenched by usual work-up and the product **2** was isolated by column chromatography. The hydrolysis in [bmim]PF_6 after 6 h yielded 80% product as against 58% yields realised in THF after 8 h. In alcoholysis strategy, 44% of **2** was isolated after 8 h in [bmim]PF_6 and the isolated yield of **2** was 50% in THF. The results obtained in the preparative experiments within practical limitations are in agreement with the HPTLC analysis.

3.4. Recycling of the lipase–ionic liquid system

To adjudge the recyclability of ionic liquid–lipase combination, we carried out the hydrolysis and alcoholysis of **1** in [bmim]PF_6 and lipase PS-C at preparative scale. After optimal time (6 h for hydrolysis and 8 h for alcoholysis) of stirring, the aqueous layer (if any) was separated and the products and unreacted substrate were extracted using diethyl ether. The ionic liquid–lipase combination was recharged with the substrate and aqueous buffer or decanol and was stirred again. Such a process was repeated twice and for each cycle, the product **2** was isolated in pure form by column chromatography. The results revealed that the yields of **2** did not vary significantly in the subsequent runs. In hydrolysis, the yields were 80, 77 and 74% in the first, second and third runs respectively; and in alcoholysis, the yields decreased only marginally from 44% in first run to 41% in the third run.

In the earlier endeavor [27], especially interesting with regard to the regiochemistry, was the deacetylation of 3,4,6-tri-*O*-acetyl-D-glucal. The *Pseudomonas fluorescens* lipase catalysed the ester cleavage in 0.25 M potassium phosphate buffer of pH 7 to yield 90% of the 3-deacetylated product. In the present study, among the three solvent combinations tried for the reaction, comparable results were observed in [bmim]PF_6-aqueous buffer solvent system, but the hydrolysis in the organic solvent, THF-aqueous buffer gave relatively less yields of the product **2**. Thus, it seems that the correct choice of the ionic liquid can mimic the natural environment (aqueous ionic medium) of the enzyme action in terms of selectivity.

4. Conclusion

In conclusion, among the solvents tried, the ionic liquid [bmim]PF_6 served as a useful reaction medium for the lipase-mediated regioselective deacetylation of 3,4,6-tri-*O*-acetyl-D-glucal via both hydrolysis and alcoholysis methodologies. On the contrary, the hydrophilic ionic liquid did not prove to be a good medium for any of the biotransformations investigated. The influence of the reaction medium on the rates and regioselectivity of enzyme catalysis has been demonstrated. The hydrolysis in ionic liquid [bmim]PF_6, proved to be the most suited protocol towards the target, 4,6-di-*O*-acetyl-D-glucal, as the advantages such as reduced reaction time, excellent regioselectivity, recyclability of the biocatalyst–solvent combination and good preparative value, were all realised together. The earlier reports by Klibanov and co-workers [34] and MacManus and Vulfson [35] have demonstrated that the preference of enzymatic deacylation towards a particular position in polyacylated substrate is dependent on the nature of organic solvents used. These observations have been rationalised by the polarity, hydrophobicity and hydrophilicity of the solvent. The explanation of the remarkable regioselectivity observed by us in the hydrophobic ionic liquid, [bmim]PF_6 is yet to be investigated.

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