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Efficient mono-acylation of fructose by lipase-catalyzed esterification in ionic liquid co-solvents

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ABSTRACT

Fructose monoesters are eco-friendly nonionic surfactants in various applications. Selective preparation of mono-acylated fructose is challenging due to the multiple hydroxyl sites available for acylation both chemically and enzymatically. Ionic liquids (ILs) have profound impacts not only on the reaction media but also on the catalytic properties of enzymes in the acylation process. In this study, utilizing an IL co-solvent system, selective synthesis of mono-acylated fructose with lauric acid catalyzed by immobilized *Candida antarctica* lipase B (CALB) was investigated. The imidazolium-based ILs selected as co-solvents with 2-methyl-2-butanol (2M2B) markedly improved the ratios of monolauroyl fructose in the presence of 60% [BMIM][TfO] (v/v) and 20% [BMIM][BF_4] (v/v), in which the mono-acylated fructose was 85% and 78% respectively. Based on a Ping-Pong Bi–Bi model, a kinetic equation was fitted, by which the kinetic parameters revealed that the affinity between fructose and acyl-enzyme intermediate was enhanced. The inhibition effect of fructose on free enzyme was weakened in the presence of IL co-solvents. The conformation of CALB binding substrates also changed in the co-solvent system as demonstrated by Fourier transform infrared spectra. These results demonstrated that the variation of CALB kinetic characteristics was a crucial factor for the selectivity of mono-acylation in ILs/2M2B co-solvents.

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1. Introduction

Sugar esters are biodegradable and nontoxic bio-surfactants which are very useful for many commercial applications. One of the attractions is fructose laurate, due to its highest cell growth inhibitory against *Streptococcus mutans* among different sugar esters.[1] Chemical synthesis tends to result in high energy cost and environmentally unsafe conditions. As a potential alternative, enzymatic preparation has mild and eco-friendly reaction conditions, as well as the inherent selectivity maintained by enzymes during reaction process.[2]

Lipases (EC 3.1.1.3) are considered as better catalysts for esterification, amidation and transesterification of esters in anhydrous media.[3] With regard to the direct esterification of fructose with lauric acid, traditional chemical reactions can result in a broad distribution of one to multi-ester species in the products. On the other hand, lipases selectively acylate the primary hydroxyl group rather than secondary groups with long chain fatty acid in anhydrous organic solvents. As the result, the products are composed of a narrow range of mono- and di-esters (Fig. 1), and it is more convenient for the separation of products. As reported, Cao's[4] and Šabeder's co-workers[5] obtained relatively higher conversions of fructose esters with lipases-mediated acylation, achieving acylation rates of 86% and 82%, respectively. However, these products were mixtures of esters in different esterification degrees, with a higher proportion of di-ester in the products. This phenomenon is considered inevitable in the direct esterification process.

Ionic liquids (ILs) have been recognized as an alternative to traditional organic solvents for the synthesis of sugar esters, not only because they offer many advantages as solvents, such as their low vapor pressure, high thermal stability and good solubilization of a wide range of carbohydrates,[6] but also as reaction media that can enhance the reactivity, selectivity, and stability of enzymes.[7–10] Several groups have reported that anhydrous ILs containing [BF_4]$^-$, [PF_6]$^-$ and [TfO]$^-$ anions can be used for biotransformation of sugars. In these ILs, desired monoacylated compounds were synthesized and/or high ester yields were achieved.[10–12]

Abbreviations: ILs, ionic liquids; 2M2B, 2-methyl-2-butanol; CALB, *Candida antarctica* lipase B; [BMIM][TfO], 1-butyl-3-methylimidazolium trifluoromethanesulfonate; [BMIM][BF_4], 1-butyl-3-methylimidazolium tetrafluoroborate; [BMIM][PF_6], 1-butyl-3-methylimidazolium hexafluorophosphate; ATR–FTIR, attenuated total reflectance-Fourier transform infrared; HPLC–ELSD, high performance liquid chromatography–evaporative light scattering detector; NMR, nuclear magnetic resonance; API–MS, atmospheric pressure ionization–mass spectrometry; ANOVA, analysis of variance.

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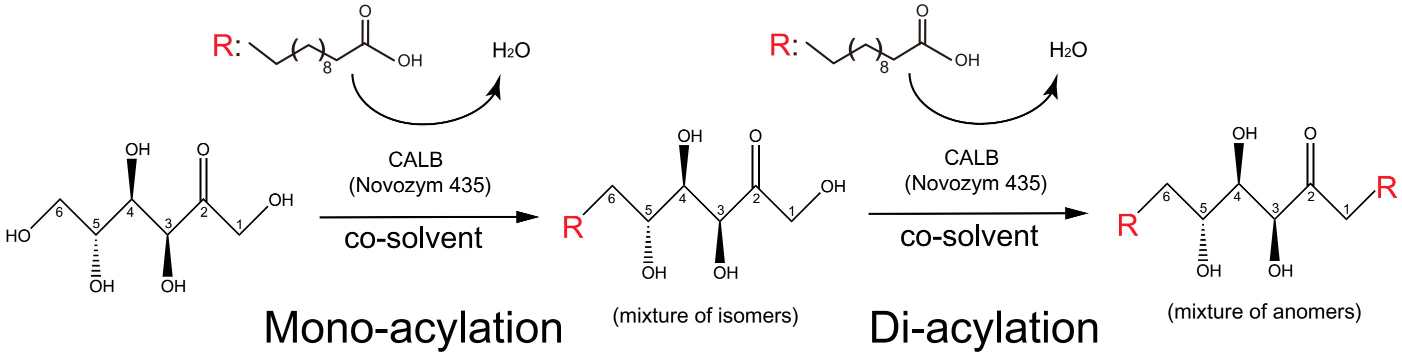

Fig. 1. Enzymatic synthesis scheme of fructose monolaurate and dilaurate.

Co-solvent system of ILs/organic solvent is a balanced approach between mass transfer limitation of pure ILs and selectivity of lipase. Our previous work revealed that in pure 2M2B, CALB displayed selectivity toward low degree of esterification products (mono-acylated fructose) and was an unfavorable solvent for di-ester synthesis.[13] Therefore, with the goal of further enhancing the selectivity and obtaining higher proportion of mono-acylated fructose laurate, our study utilized three imidazolium-based ionic liquids ([BMIM][BF_4], [BMIM][PF_6] and [BMIM][TfO]) to constitute an ILs/2M2B co-solvent system as the reaction media in the acylation process of fructose.

To probe the key factors that affect the activity and selectivity in mono-ester synthesis, we first experimentally surveyed the productivity of fructose laurate and the selectivity of mono-acylation in different volume ratios of the ILs/2M2B co-solvent system. Then the optimal reaction media were further studied for the characteristics that are particularly related to the solubility of substrate, mass transfer limitation, and the kinetics and structure variation of CALB.

2. Results and discussion

2.1. *Effect of volume ratios in the ILs/2M2B co-solvent system*

For the enzymatic direct esterification of fructose with lauric acid, high conversion rate was obtained by the approach of providing excessive insoluble fructose in a small amount of organic solvent-adjuvant.[4,5] Based on this protocol, we set about conducting the reaction process in co-solvents of ILs/2M2B. In our case, we expected an increase of mono-acylated product in such reaction media. Having developed an optimized co-solvent system, the synthesis process was launched at different volume percentages of ILs in 2M2B. For comparison, the synthetic reaction was also assayed in pure 2M2B and pure ILs.

HPLC and mass spectrometry revealed that, within the reaction time of 12 h in either pure 2M2B or co-solvent system, only mono-acylated fructose laurate and di-acylated fructose laurate were detectable in the products. The structures of products were further confirmed by ^{13}C NMR spectroscopy, a method employed in previous reports.[14,15] We found that mono-ester was a mixture composed of 1-α-fructofuranose laurate, 1-β-fructopyranose laurate, 6-lauroyl-α-D-fructofuranose and 6-lauroyl-β-D-fructofuranose; the di-esters were composed of 1, 6-dilauroyl-α-D-fructofuranose laurate and 1, 6-dilauroyl-β-D-fructofuranose laurate.

As shown in Fig. 2, the addition of ionic liquids resulted in fluctuation in the yields of products and especially the increased selectivity of mono-acylation. The proportion of mono-acylated product had a clear improvement ($P < 0.05$) in the [BMIM][BF_4]/2M2B and [BMIM][TfO]/2M2B co-solvent system. As shown in Fig. 2A, we found that with the increase of [BMIM][BF_4] component, yield and selectivity of mono-acylation content were gradually increased, beyond which further increase in IL content resulted in a substantial decline. At the point of 20% [BMIM][BF_4], we detected

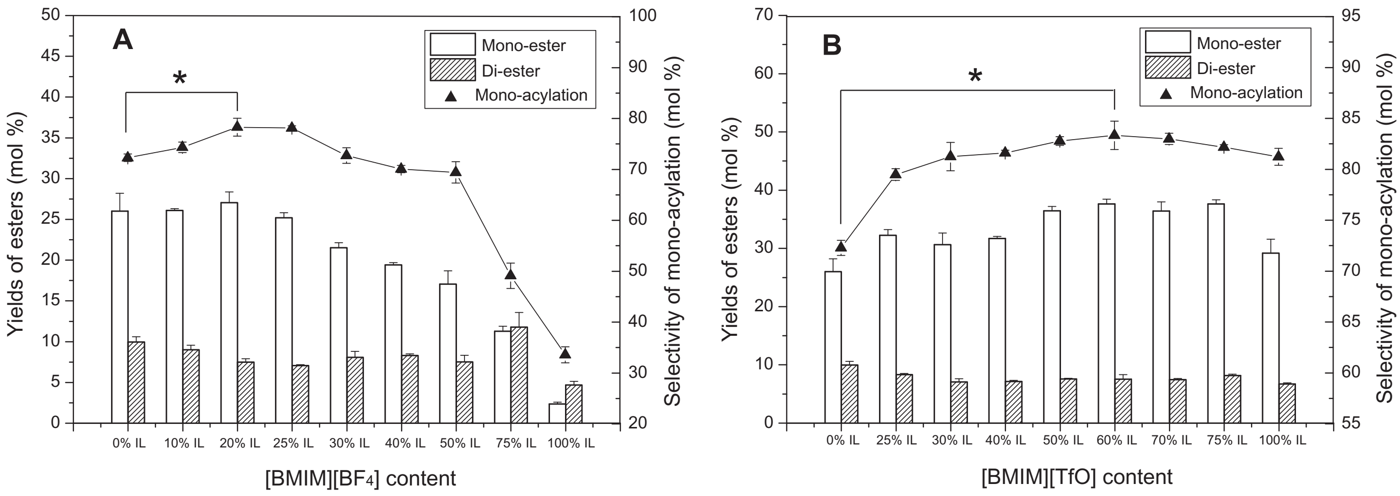

Fig. 2. Effects of volume ratios on esterification in ILs/2M2B co-solvent system. Different ratios of [BMIM][BF_4] (A), [BMIM][TfO] (B) as co-solvent in 2M2B (v/v). Reaction conditions: 0.222 mmol fructose, 0.222 mmol lauric acid, 0.6 mL reaction media, 25 mg immobilized CALB, 100 mg molecular sieve (3 Å), 50 °C, 200 rpm (orbital stirrer), 12 h. Asterisks indicate significant differences ($P < 0.05$).

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