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Ionic liquids as novel solvents for biosynthesis of octenyl succinic anhydride-modified waxy maize starch

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ABSTRACT

Biosynthesis of octenyl succinic anhydride (OSA) starch was investigated using ionic liquids (ILs) as reaction media. Waxy maize starch was pretreated in 1-butyl-3-methylimidazolium chlorine and then esterified with OSA in 1-octyl-3-methylimidazolium nitrate by using Novozyme 435 as catalyst. The degree of substitution of OSA starch reached 0.0130 with 5 wt% starch concentration and 1 wt% lipase dosage based on ILs weight at 50 °C for 3 h. The formation of OSA starch was confirmed by fourier transform infrared spectroscopy. Scanning electron microscopy and X-ray diffraction revealed that the morphology and crystal structure of starch were significantly destroyed. Thermogravimetric analysis showed that esterification decreased the thermal stability of starch. The successful lipase-catalyzed synthesis of OSA starch in ILs suggests that ILs are potential replacement of traditional organic solvents for starch ester biosynthesis.

1. Introduction

Starch is readily available, renewable, nontoxic and biodegradable, and its physicochemical properties are usually modified to better suit the industry requirements [1,2]. Currently, starch is modified either by esterification, polymer grafting, etherification, hydrolysis or hydrothermal treatments [3,4]. Among the modified starches, starch ester is extensively applied as thickener, stabilizer, emulsifier, and foaming agent in foods and other fields including textiles, pharmaceuticals and cosmetics industries [1,5]. Starch esterification has been extensively investigated. However, esterification of native starch remains challenging because of its low solubility in most conventional solvents. The few exceptions, such as dimethyl sulfoxide, *N*,*N*-dimethylacetamide and pyridine, are unfortunately volatile and toxic [6]. The extensive use of these solvents renders esterification dangerous and increases the risk of environmental pollution.

Ionic liquids (ILs), which are lowly toxic, nonflammable and recyclable, as well as possess considerably low vapor pressures, are potential solvents [7–9]. With the discovery of ILs, a new starch modification method is introduced [10,11]. ILs based on anions of formate, nitrate, acetate, sulfate, halogen and dicyandiamide have been confirmed as excellent starch solvents [12–18]. The solubility of starch in 1-butyl-3-methylimidazolium chloride ([BMIm]Cl) and 1-allyl-3-methylimidazolium chloride reaches 10 wt% and 15 wt%, respectively [19,20]. Furthermore, several kinds of starch esters, such as starch laurate, starch stearate, starch succinate, starch phosphate and starch acetate, have been chemically prepared using ILs as reaction media in recent years [6,21–24]. However, research on enzymatic modification of starch in ILs has rarely been conducted. The possible reason for this phenomenon is that starch-dissolving ILs are more likely to denature enzymes because of their strong polarity, thereby preventing further biotransformation of the starch dissolved in ILs [25–27].

To explore suitable ILs media for enzymatic reaction, various efforts have been made. Zhao et al. [27] provided a platform to examine the relationship between the IL structure and both the carbohydrate solubility and enzyme activity in ILs. ILs based on hydrophilic anions are good solvents for carbohydrate dissolution, but may also inactivate enzyme. Grafting a long side chain onto the IL cation can reduce the relative concentration of denaturing anion in IL and thus benefits to enzyme activity, which is consistent with the result obtained by Li Na et al. [25]. Li Na et al. investigated the effect of 1-alkyl-3-methylimidazolium ILs on the hydrolysis activity of *Candida rugose* lipase toward triacylglycerol and the results showed that ILs with longer alkyl chain in cation $[C_n\text{MIm}]^+$ could achieve higher enzyme activity. Therefore, 1-octyl-3-methylimidazolium nitrate ($[\text{OMIm}]NO_3$) was chosen as reaction

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medium for starch bioesterification in this study. The anion of NO_3^- contributed IL to form hydrogen bonds with the hydroxyl groups of starch molecules, which increased starch solubility. A long side-chain attached to the IL cation induced a low NO_3^- concentration in this IL, thus rendering the IL more favorable to enzyme activity.

Furthermore, Lu et al. reported a two-step method that effectively enhanced the bioreactivity of starch esterification [24]. The two-step method can be described concisely as follows. Starch was initially dissolved in [BMIm]Cl and then esterified with an esterifying agent in [BMIm]BF_4. The predissolved step destroyed the granular structure of starch, thereby rendering the starch chains more accessible to reagents, especially the lipase molecule. The two-step method separated the dissolution process from the esterification process, thus resulting in high reactivity.

Therefore, a two-step method was applied to prepare OSA starch in this study. Starch was prepared in [BMIm]Cl at 100 °C for 2 h. Thereafter, the pretreated starch was esterified with OSA (3 wt%, based on the dry weight of starch) in [OMIm]NO_3 with Novozyme 435 as biocatalyst. The reaction conditions, including starch concentration, lipase dosage, reaction temperature and reaction time, were optimized. The obtained starch esters were characterized by fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM) and thermogravimetric analysis (TGA).

2. Materials and methods

2.1. Materials

Waxy maize starch was purchased from Binzhou JinHui Corn Development Co., Ltd. (Shandong, China). The starch was dried at 60 °C for 48 h before use but without further purification. OSA was purchased from Deqing SanFu Food Co., Ltd. (Huzhou, China) and was of food grade. Novozyme 435 (*Candida antarctica* lipase B) was acquired from Sigma Chemicals (St. Louis, MO, USA). [BMIm]Cl (>99%) (Scheme 1) and [OMIm]NO_3 (>99%) (Scheme 2) were obtained from ChengJie Development Co., Ltd. (Shanghai, China). All other analytical-grade chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (Suzhou, China).

2.2. Determination of starch solubility in [BMIm]Cl

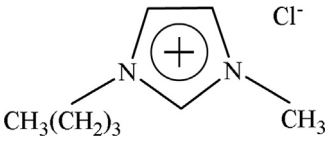
Dried waxy maize starch was added incrementally (25 mg) into [BMIm]Cl (10 g). After adding the first increment, the mixture was heated at 100 °C with continuous stirring for 2 h. If starch slurry was transparent and starch granules exhibited no polarized cross, the next increment was added. The saturation limit reached when starch slurry became turbid and polarized cross did not vanish after 2 h of adding sample.

2.3. Pretreatment of starch in [BMIm]Cl

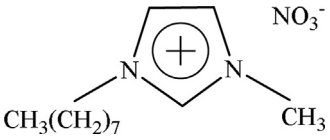
Starch was predissolved by mixing dried starch with [BMIm]Cl at 10 wt% concentration and heating at 100 °C while shaking for 2 h in an oil bath. After cooling to about 60 °C, starch was isolated by adding anhydrous ethanol (100 mL) to the mixture, followed by centrifugation at 6400 × *g* for 20 min. The precipitation was then Soxhlet extracted with anhydrous ethanol at 75 °C for 24 h to remove the residual IL [BMIm]Cl and finally dried under vacuum at 40 °C for 48 h. The obtained samples were used in biomodification experiments.

Table 1
Solubility of normal corn starch in [BMIm]Cl at different temperatures.

Temperature (°C)	70	80	90	100	110	120
Solubility (wt%)	5	8	10	12	14.25	16



Scheme 1. Structural scheme of 1-butyl-3-methylimidazolium chlorine ([BMIm]Cl).



Scheme 2. Structural scheme of 1-octyl-3-methylimidazolium nitrate ([OMIm]NO_3).

2.4. Lipase-catalyzed synthesis of OSA starch in [OMIm]NO_3

Waxy maize starch was added into [OMIm]NO_3 at the required temperature for 1 h with continuous stirring. Subsequently, OSA and Novozyme 435 were added into the mixture according to the experimental design, and then the mixture was heated at the desired temperature for a certain period. At the end of the reaction, starch and its ester were precipitated using sufficient anhydrous ethanol. The precipitate was subsequently separated from the IL by centrifugation (6400 × *g*, 20 min). To remove the residual IL, unreacted reagents, and byproducts, we thoroughly washed the precipitate through Soxhlet extraction for 24 h (anhydrous ethanol) and finally dried at 40 °C for 48 h.

2.5. Determination of degree of substitution (DS)

DS value of OSA starch was determined according to the published titration method with a slight modification [28,29]. Dried OSA starch (0.5 g) was dispersed in 2.5 mL of 2.5 mol/L hydrochloric acid–isopropyl alcohol solution with rapid stirring for 30 min, followed by adding 10 mL of 90% (v/v) isopropyl alcohol solution and stirring for additional 10 min. Subsequently, the suspension was filtered under vacuum, and the residue was thoroughly washed with 90% (v/v) isopropanol solution until Cl^- was no longer detected in the effluent (using 0.1 M silver nitrate solution). The filter mass was re-dispersed in 30 mL of distilled water and then vigorously stirred in a boiling water bath for 10 min. The resulting starch solution was titrated with 0.1 M sodium hydroxide (NaOH) solution using phenolphthalein as an indicator. The DS of OSA starch was calculated using Eq. (1):

$$\mathrm{DS} = \frac{162 \times (C \times V)/W}{1000 - 210 \times (C \times V)/W} \quad (1)$$

where 162 is the molecular weight (g/mol) of anhydrous glucose unit (AGU), 210 is the molecular weight (g/mol) of OSA, V is the titration volume (mL) of NaOH solution, C is the concentration (mol/L) of NaOH solution, and W is the dry weight (g) of OSA starch.

The reaction efficiency (RE) was calculated using Eq. (2):

$$\mathrm{RE} = \frac{\text{Actual DS}}{\text{Theoritical DS}} \quad (2)$$

where theoretical DS was calculated by assuming that all of the added anhydride reacted with starch to form the ester derivative [1].