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A kinetic study of starch palmitate synthesis by immobilized lipase-catalyzed esterification in solvent free system

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ABSTRACT

The objective of this work was to propose a reaction mechanism and to develop a rate equation for the synthesis of starch palmitate by acylation of the corn starch with palmitic acid using the lipase Novozym 435 in solvent-free system. Initial rate data and progress curve data were used to arrive at a suitable model. The initial rate studies showed that the kinetics obey the Ping-Pong bi-bi mechanism. An attempt to obtain the best fit of this kinetic model through computer simulation yielded in good approximation, the kinetic equation was $v=(1.735\times C_{\text{fatty-acid}}\times C_{\text{starch}})/(C_{\text{fatty-acid}}\times C_{\text{starch}}+0.0156\times C_{\text{starch}}+2.3947\times C_{\text{fatty-acid}})$. The mathematical expressions have been tested using several sets of data obtained from reactions carried out under different reaction conditions. The predicted values provide very good fits of the experimental data for the molar of starch from 2 mmol to 10 mmol, the molar of palmitic acid from 5 mmol to 70 mmol, the reaction temperature from 50 °C to 70 °C, amount of lipase from 44 mg to176 mg, rotate speed from 100 r/min to 240 r/min, initial a_w from <0.01 to 0.57.

1. Introduction

Starch is an abundant renewable polysaccharide in nature that is inexpensive, fully biodegradable and widely used in the production of both food and industrial products [1,2]. Chemical modification starch is often required to better suit its properties to specific applications. Many reports exist in literature pertaining to the preparation of starch esters or its components with the ultimate aim of significantly modifying the physical–chemical properties of starches and imparting suitable mechanical characteristics so as to render them more useful as engineering materials than native starch [3,4].

Interest in an enzymatic route to esterify starch is fairly recent and most works have been published after 2005 [5], with the exception of one earlier investigation. A number of groups have recently reported the use of organic solvents for esterification of starch [6]. Normally, dimethyl sulfoxide (DMSO), dimethyl formamide (DMF) and pyridine are used to dissolve the starch to make it more reactive toward esterification [7]. Some authors [8] have reported the preparation of a high degree of starch esters in the presence of organic solvents using microwave heating. Unlike chemical esterification modification, an enzymatic one is an environmentally friendly method which occurs under milder conditions. The use of lipase as catalyst for ester production has great potential. In fact, using a biocatalyst eliminates the disadvantages of the chemical process by producing very high purity compounds with fewer or no downstream operations [9,10].

Although the introduction of an ester group into starch is an important chemical modification task [11], little information is available about the kinetic models and their parameters. Most of the lipase kinetic studies are relative to hydrolysis reactions, while the esterification kinetic publications are quite rare [12]. Some of the models proposed for ester synthesis consider a simple Michaelis–Menten mechanism, but are only valid for the simplest enzymatic reactions. However, most approaches have proposed a Ping-Pong Bi-Bi mechanism which seems to give the best results in reproducing experimental findings [12–14].

In a previous paper [15] we have studied the influence of the acyl donor, granule shape and crystal structure of corn starch and the type of enzyme, as well as the main operating parameters [16], in the enzymatic production of starch ester. The best yields were obtained when using palmitic acid as acyl donor, pretreatment starch by sodium hydroxide/urea aqueous solution and the commercial immobilized lipase Novozym 435 as catalyst in solvent free system.

The aim of this work was to conduct a kinetic study of the enzyme synthesis of starch palmitate in solvent free system. With that purpose, it was first carried out a deep study of the reaction

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and the inhibition effect of substrates and products was also investigated since this phenomenon is quite often in enzymatically catalyzed reactions.

2. Material and methods

2.1. Chemicals and enzyme

Corn starch was purchased from Harbin Mei Wang Reagent Company, China and pretreatment by our laboratory. Palmitic acid of analytically grade was purchased from Shanghai Chemical Co., China. Novozym 435 (Lipase B from Candida Antarctica immobilized on macroporous acrylic resin; specific activity: 10,000 U/g) was purchased from Novozymes, Denmark. All the other chemicals are of analytically grade.

2.2. Starch pretreatment

According to [17] the 9% aqueous solution containing sodium hydroxide/urea at the desired ratio of 2:1 by weight was used as a solvent for starch. The solvent was pre-cooled to below −10 °C. Then the starch sample in the given amount of 5% was added immediately at ambient temperature of below 25 °C. The native starch (NS) was completely dissolved within 5 min by stirring at 3000 r/min and the resultant solution was transparent. The transparent starch solution was neutralized with HCl (15%) until it reached neutrality. Then, starch was precipitated out from the neutral starch solution by adding 50 mL of ethanol drop-wise. After various durations of dropping treatment, the precipitates were washed by successive centrifugations in 95% of ethanol until no HCl remained. Thereafter, they were washed with 100% of ethanol to remove water. The resulting precipitates were vacuum dried at 50 °C for 24 h.

As show in our previous studies [16,18], the average particle size of starch decreased to nanometer level from 4 μm to 15 μm after pretreatment. The crystalline type of corn starch shift from A-type to V_H-type and the relative degree of crystallinity of corn starch had been decreased to 10.32%. The smaller particle size and the destruction of the crystal structure of starch after pretreatment endowed starch with higher cold-water solubility. The esterification activity of corn starch had been significantly improved after pretreatment.

2.3. Water activity pre-equilibration of reaction medium

Before the start of the reaction, the substrates (palmitic acid, pretreatment starch and Lipase) were pre-equilibrated for at least 3d in a sealed containers enclosed with saturated salt solutions or solid adsorbent to establish fixed water activities for esterification. Pre-equilibration was done at 25 °C. The solid adsorbent was 3 Å molecular sieves ($a_w < 0.01$). The saturated salt solutions used were prepared with LiBr (a_w: 0.05), LiCl (a_w: 0.11), CH_3COOK (a_w: 0.23), $(MgNO_3)\cdot 6H_2O$ (a_w: 0.54), NaCl (a_w: 0.75), KCl (a_w: 0.85), $K_2Cr_2O_7$ (a_w: 0.98) [19].

2.4. General procedure for lipase esterification

Water activity or a_w is an important consideration for biocatalysis in a solvent free medium. Before the start of reaction, all the substrates were pre-equilibrated for at least 3d in sealed containers, enclosed with a molecular sieve to establish fixed water activities ($a_w < 0.01$). The reaction setup for esterification was carried out in 25 mL closed, screw-capped glass vials containing palmitic acid and pretreated starch. To conduct the reaction under neat conditions (without solvents), a 5:1 mol ratio of palmitic acid to pretreated starch is needed to provide enough solution volume to dissolve solid starch and to stir the suspended immobilized lipase. The palmitic acid acted as the solvent in the solvent free system when the reaction temperature was above of its melting point (63–64 °C). The esterification was initiated by adding immobilized lipase (Novozym 435) into each glass vial. Glass vials were placed upright on a magnetic stirrer and incubated at 55–75 °C, 40–240 r/min for 4–24 h. The removal of nonesterified palmitic acid from starch palmitate was accomplished by washed again with 100 mL of pure ethanol and then dried in a hot air oven at 75 °C.

2.5. Calculation of the Initial reaction rates

Initial reaction rates, expressed as m mol consumed palmitic acid per minute and per gram of enzyme, were determined from the time course of palmitic acid concentration. In order to get the parameters of the kinetic model, initial velocities were fitted to the proposed reaction rate equation by non-linear regression analysis with the computer program Microsoft Matlab.

2.6. Calculation of the conversion of palmitic acid

A small sample 30 mg of starch palmitate dissolved in 1 mL DMSO was mixed with 1 mL of sodium methoxide (0.07 M) in methanol solution. This mixture was then heated (70 °C) under reflux for 40 min, while shaken, then cooled and 1 mL of deionized water and 1 mL of *n*-heptane were added. The mixture was shaken for 1 min and left to settle. The top organic phase contained the methyl ester of palmitic acid and could be removed and injected into the GC–FID (Perkin-Elmer Autosystem XL with a CP Simdist capillary column, oven set at 220 °C, the injector at 250 °C and the detector at 260 °C, flow rate of N_2 and air is 4.5 mL/min and 5.5 mL/min, flow rate of tail-blowing is 5.0 mL/min).

Once the methyl oleate was quantified by GC chromatograph, the conversion of palmitic acid (CP) was calculated as Eq. (1).

$$CP = \frac{M_1}{M_0} \times 100\% \quad (1)$$

where CP is the conversion of palmitic acid; M_0 is the initial mole of palmitic acid, mol; M_1 is the mole of esterified oleic acid, mol.

3. Results and discussions

The effect of various parameters on the rate of reaction were studied to arrive at a suitable kinetic model.

3.1. Effect of different catalysts

Various catalysts such as Candida cylindracea lipase (CRL), Porcine pancreas lipase (PPL), Immobilized thermophilic fungal lipase (TLIM), Novozym 435 (Candida Antarctica lipase immobilized on a macroporous polyacrylic resin) were tested (Fig. 1).

The enzyme activity per mg enzyme was different in each case. Esterification activity of various lipases was determined by a reported esterification method [20]. The unit of enzyme activity is defined as μmol of palmitic acid consumed (in an esterification reaction with pretreatment starch) per min per mg of the enzyme (Table 1).

Of this Porcine pancreas lipase (PPL) and Candida cylindracea lipase (CRL), led to poor conversions of 3% and 7% in 24 h, respectively, while Immobilized thermophilic fungal lipase (TLIM) offered comparable conversions around 23% in 24 h. Novozym SP 435 was found to be the best catalyst with a conversion of 57% in 24 h. Generally Immobilized thermophilic fungal lipase (TLIM) is very active on long chain fatty acids. However, in the case of starch palmitate synthesis it was less effective. Candida cylindracea lipase (CRL) and Porcine pancreas lipase (PPL) has been reported to be a very good