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Lipase-catalyzed acylation of konjac glucomannan in organic media

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Abstract

Lipase-catalyzed acylation of konjac glucomannan (KGM) with vinyl acetate in organic media was successfully conducted using Novozym 435 as a biocatalyst. The degree of substitution (DS) of the modified KGM was used to evaluate the extent of acylation. The influences of various factors, such as water activity (a_w), organic media, reaction temperature, shaking rate, enzyme dosage and the molecular weight of KGM, on the reaction were investigated. The water activity of the reaction system played a key role in the acylation of KGM. *tert*-Butanol (*t*-BuOH) was thought to be the most suitable reaction medium by taking DS of the modified KGM into account. The optimum water activity, shaking rate, reaction temperature and enzyme dosage were 0.75, 200 r/min, 45–50 °C and 250 U/ml, respectively, under which the DS of product was as high as 0.71. It has also been found that the DS of modified KGM sample decreased with increasing molecular weight of KGM. Additionally, Novozym 435-catalyzed acylation of KGM was proved to be highly regioselective, with C6-OH being acylated.

Keywords: Konjac glucomannan; Lipase; Acylation; Degree of substitution; Water activity; Organic media

1. Introduction

Konjac glucomannan (KGM) is a natural polysaccharide isolated from the tubers of *Amorphophallus konjac* plants. It consists of β-1, 4-linked D-glucose and D-mannose units, and the molar ratio of glucose and mannose has been reported to be around 1–1.60 (Fig. 1) [1–3]. There may be certain short side branches at the C-3 position of the mannoses and acetyl groups randomly present at the C-6 position of a sugar unit [1,3]. The acetyl groups frequently range from 1 per 9 sugar units to 1 per 20 sugar units [1,4]. Furthermore, there may be some differences in molecular structure of KGM from different species [2,5]. KGM possesses excellent biodegradability, biocompatibility, and many unique pharmacological functions.

Chemically modified forms of KGM have been studied and shown to be promising as a novel medicine [6], an environmentally benign emulsifier [7] and drug carriers [8]. KGM can participate in chemical reactions to form their derivatives because of the presence of the hydroxyl groups on sugar units. But they are usually difficult to be selectively modified. Typically, their selective chemical modification requires protecting and de-protecting steps that are time-consuming and expensive. Moreover, the catalysts and reagents used for the chemical modification of KGM may cause safety concerns if the products are intended for use in the cosmetic, food and pharmaceutical industries. An alternative route is a biocatalytic process, which is not only facile and green, but could lead to highly selective modification of KGM.

It is well known that enzymatic ester synthesis is thermodynamically unfavorable in conventional aqueous media [9]. Methods have been developed to facilitate the formation of ester bonds over their hydrolysis. One approach is to replace water with an organic solvent to favor synthesis under restricted water conditions [9]. Another is using an activated ester, usually a vinyl ester, as an acyl donor. Vinyl esters are known for their high reactivity in enzyme-catalyzed esterification or transesterification reactions and the vinyl alcohol formed during the reaction tautomerizes to acetaldehyde, thereby making the reaction irreversible (Scheme 1). Up to now, there has been little study on enzymatic modification of polymers and nothing has been known about enzymatic acylation of KGM. In this paper, the possibility of acylation of KGM with vinyl acetate via immobilized lipase Novozym 435 in organic media for the preparation of KGM esters was explored. The effects of some major influential factors, such as initial water activity (a_w), shaking rate, reaction temperature,

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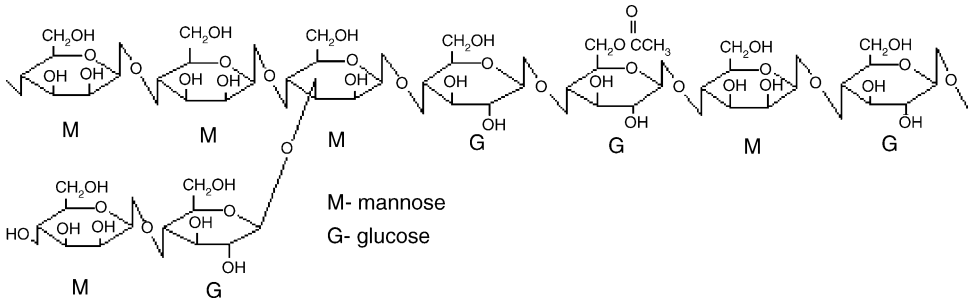


Fig. 1. Molecular structure of KGM.

enzyme dosage and the molecular weight of KGM, on the reaction were examined.

2. Materials and methods

2.1. Materials

KGM (M_w = 77,503, M_w/M_n = 3.4) used as a substrate was prepared by enzymatic degradation of a native KGM powder (M_w = 980,000, M_w/M_n = 1.7; a donation of Multi-Ring Health Products, Ltd., China) [10]. KGM was milled into a powder prior to use. The KGM powder passing through a 120-mesh sieve was used in the reactions. Novozym 435 (Lipase B from *Candida antarctica* immobilized on macroporous acrylic resin; specific activity: 10,000 U/g) was purchased from Novozymes, Denmark. Vinyl acetate, dimethylsulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), pyridine, *tert*-butanol (*t*-BuOH), toluene and isooctane were purchased from Shanghai Chemical Co., China and are of analytical grade.

2.2. Analytical methods

The molecular weight and molecular weight distributions M_w/M_n of KGM samples were measured by GPC using a commercial GPC system (Waters 515, Waters Co.) equipped with a column (Ultrahydrogel 500, Waters Corp., Milford, MA, USA), a pump (Waters 515) and a Waters 2410 refractive index detector. Dextran was used as a standard for calibration. The mobile phase was pure water at a flow rate of 0.6 ml/min.

SEM micrographs were obtained with a Quanta 400 scanning electron microscope (FEI Company, Holland) at an accelerating voltage of 20 kV. FTIR spectra were recorded using a Vector 33 spectrometer (Brucker Company, Germany). Potassium bromide (KBr) disks were prepared from powdered samples mixed with dry KBr in the ratio of 1:100. The spectra were recorded in a transmittance mode from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. The ^{13}C NMR spectra were recorded at room temperature in DMSO-d_6 by using tetramethylsilane (TMS) as an internal reference on a Brucker DRX-400 NMR spectrometer (Germany) at 100 MHz.

2.3. Determination of degree of substitution (DS)

The degree of substitution of modified KGM products was determined by means of titration method with some modifications [11]. Briefly, dried sample of powered KGM acetate (0.10 g) was placed in a 100 ml Erlenmeyer flask with a stopper and 75% ethanol (10 ml) was added. The mixture was stirred at 50 °C for 30 min, and cooled to room temperature, followed by adding 0.5N KOH (5 ml) with swirling. Then the flask was kept at 30 °C for 72 h with stirring. The excess alkali was back titrated with 0.1N hydrochloric acid using phenolphthalein as an indicator. A blank (to which no KGM acetate sample had been added) was titrated in parallel. Eq. (1) was used to calculate the content of acetyl groups in the sample:

$$\%\,\text{acetyl}\,(\text{w/w}) = \frac{(V_a - V_b) \times N_{HCl} \times M_{acetyl}}{m_s} \times 100 \quad (1)$$

where V_a is the volume of hydrochloric acid consumed for the blank in liters, V_b represents the volume of hydrochloric acid consumed for the sample in liters, N_{HCl} stands for the normality of the hydrochloric acid, M_{acetyl} = 43 g/mol and m_s is the mass of the sample in grams.

The DS can be calculated according to Eq. (2). The average error for DS determination is less than 1.0%. All reported data are averages of experiments performed at least in duplicate:

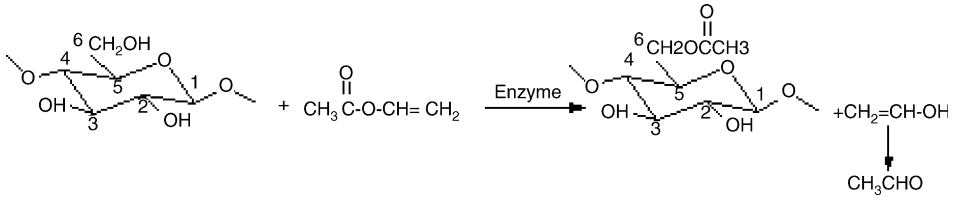
$$\text{DS} = \frac{162 \times \%\,\text{acetyl}}{4300 - 42 \times \%\,\text{acetyl}} \quad (2)$$

2.4. Water activity control

Organic solvents and the substrate were equilibrated to fixed water activities (a_w) over saturated salt solutions in closed container at 25 °C. The following salts were used: LiCl (a_w = 0.11), $MgCl_2$ (a_w = 0.33), $Mg(NO_3)_2$ (a_w = 0.54), NaCl (a_w = 0.75), KCl (a_w = 0.84) and K_2SO_4 (a_w = 0.97) [12]. The equilibration was monitored by water analysis using Karl Fischer titration until constant water contents were observed. The enzyme was equilibrated in separate vessels at 25 °C.

2.5. General procedure for enzymatic acylation of KGM

Vinyl acetate was used as an acylating agent for lipase-catalyzed modification of KGM (Scheme 1). A typical enzymatic acylation reaction was carried out in 10 ml of organic solvent with specified a_w value containing 0.4 g of KGM (2.5 mmol of sugar units) and 7.5 mmol of vinyl acetate by the addition of Novozym 435 in an air-bath shaker under predetermined reaction conditions. The reaction was terminated by filtering off the enzyme. Organic solvent was removed by distillation under reduced pressure. The product was washed thoroughly with ethanol and then dried under vacuum. Control reaction, which was performed following the above procedure except that no enzyme was added, demonstrated that no chemical acylation of KGM was detectable.



Scheme 1. Enzyme-catalyzed acylation of KGM with vinyl acetate.