Preliminary Study on the Preparation of Esterified Konjac Glucomamman by Lipase-Catalyzed Reactions in Non-aqueous Systems*

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Abstract: Enzyme-catalyzed esterification and transesterication of Konjac glucomannan (KGM) in different non-aqueous reaction systems with different lipases as a biocatalyst were investigated in order to seek a new feasible and environmentally benign way by which the esterified KGM derivatives can be prepared. The preliminary experimental results show that the esterified derivatives of KGM can be prepared by lipase-catalyzed reactions in non-aqueous systems. Lipase-OF and Lipozyme Tl 100L are effective biocatalysts in the preparation of esterified KGM under proper conditions.

Key words: konjac glucomannan; lipase; biocatalysis; non-aqueous system; esterification
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1 Introduction

Konjac glucomannan (KGM) is the main component found in tubers of the Amorphophallus konjac plant and it constitutes 60% ~ 80% of the dried tuber[1]. It is abundant in nature. The plant has been cultivated in China, Japan and the other countries in the Far East for many centuries and it is used in traditional Chinese and Japanese cooking to make noodles and gels which are stable in boiling water. KGM is a linear random copolymer composed of β-1,4-linked *D*-mannose and *D*-glucose chains in a molar ratio of 1.6 to 1 or 1.69 to 1, depending on the origin of KGM[2-4]. There are 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[3,5], as shown in the chemical structure in Fig. 1. The acetyl groups frequently range from one per six sugar units to one per twenty sugar units[5]. The degree of water solubility is controlled by the presence of the acetyl units.

KGM is usually used as a food additive due to its strong thickening effect and good gelling properties. In addition, it is known to interact synergistically with xanthan gum, kapa carrageenan and starches, enhancing their rheological properties. As a natural polysaccharide, KGM possesses excellent biodegradability, biocompatibility and many unique physiological and pharmacological functions. It is interesting that KGM and its derivatives have been found to have some unique biological activities that are very useful for biomedical application. For instance, some studies have proved that KGM is effective in preventing hypercholesterolemia[6], inhibiting tumours[7-9], and decreasing blood lipid and serum cholesterol levels[10], and its carboxylated derivative has strong immunological enhancement activity[11].

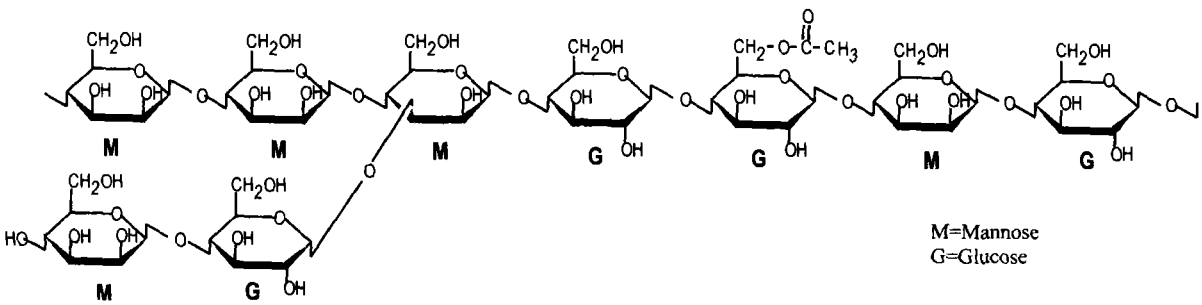

Fig. 1 The macromolecular structure of KGM

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It has been reported that some chemically modified KGM products can be used as a novel medicine[12] and environmentally benign chemicals such as an emulsifier[13] and a flocculant[14].

However, all the KGM derivatives were prepared by means of traditional chemical modifications, which are often hazardous and environmentally unfriendly. They have to consume much more chemicals than actually needed for a reaction and cannot inhibit side reactions from happening, leading to big difficulties in finely controlling the structure of products and purifying products. Therefore, it is of great interest to modify a polysaccharide using the alternative way green chemistry. Based on this consideration, we make an attempt to prepare one of KGM derivatives, an esterified product of KGM, by use of a biocatalyzed reaction that is environmentally friendly. As a part of this research project, enzyme-catalyzed degradation of KGM has been studied[15,16]. The present work involves enzyme-catalyzed esterification and transesterication of KGM in different non-aqueous reaction systems with different lipases as a biocatalyst to seek a new feasible and environmentally benign approach to prepare esterified KGM derivatives. Some preliminary experimental results are reported here.

2 Experimental

2.1 Materials and chemicals

Original Konjac glucomannan (KGM), purified powder, was a gift from Multi-Ring Health Products, Ltd., China. The lipases used in the experiments were Lipase-OF (Meito Sangyo Co., 900 units/mg), Lipase-MY (LTD Co., 950 units/mg), Lipase-me (Novo Nordisk Co., 400 units/mg), Novozyme® 435 (Novo Nordisk Co., 950 units/mg), Candida rugosa Lipase (Sigma Co., 890 units/mg) and Lipozym Tl 100 L (Novo Nordisk Co., 100 KLU/g). The other chemicals were analytical grade reagent and used as purchased.

2.2 Esterification of KGM

Enzymatically degraded KGM samples with relatively low molecular weight (< 30 000) prepared by a method described in previous work[16] were used as substrates in the preparation of esterified KGM samples. Esterification of KGM can be realized through two ways. One is a direct esterification reaction using an acid anhydride or a carboxylic acid as an acyl donor, and the other is a transesterification reaction using a caboxylic ester as an acyl donor. Both the reactions were conducted at 44 ℃ in an organic solvent or in a reversed micellar system for 48 hours. A sufficient amount of ethanol was added to end the reaction. Esterified products were repeatedly washed with ethanol followed by lyophilization.

2.3 Characterization

The degree of esterification can be roughly characterized by the amount of consumed acyl donor. The amount of consumed acyl donor was determined by an acid-base titration in a non-aqueous medium[17]. Infrared spectra of KGM and esterified KGM samples were recorded with a Fourier transform IR (FTIR) spectrometer (FTS3000, Bio-Rad Co, USA). KBr pellets were made.

3 Results and discussion

The results of lipase-catalyzed esterification of KGM in organic solvents are shown in Table 1. When succinic anhydride was used as an acyl donor, the results seemed to indicate that these lipases can catalyze the esterification of KGM to some extent except Candida rugosa lipase among which Lipase-OF, Lipase-MY and Lipase-me

Tab.1 The results of lipase-catalyzed esterification of KGM in organic solvents

No	Sample	Substrate	Reaction medium		Acyl donor		Lipase		Consumed
		mg	Solvent	V/mL	Reagent	Amount	Commodity name	m/mg	Acyl Donor/%
1	Ea1	300	DMF	10	Succinic	400 mg	Lipase-OF	40	3.75
2	Ea2		FA	10	Anhydride		Lipase-OF	30	11.00
3	Ea3		FA	40			Lipase-MY	50	8.74
4	Ea4		FA	40			Lipase-me	60	7.50
5	Ea5		FA	40			Novozyme® 435	50	5.00
6	Ea6		FA	40			Lipase-OF	50	3.75
7	Ea7		FA	40			*Candida rugosa* lipase	50	0
8	Eb1	100	–	–	Formic	10 mL	Lipase-OF	50	4.9
9	Eb2		–	–	Acid		Lipase-me	50	5.6
10	Eb3		–	–			Lipase-MY	50	4.4
11	Eb4		–	–			Novozyme® 435	60	5.0
12	Eb5		–	–			*Candida rugosa* lipase	50	4.9

exhibit relatively high catalytic activity. In addition, formamide (FA) is more favorable to the esterification of KGM than N, N-dimethylformamide (DMF). In Tests No. 8 – 12 formic acid was used not only as an acyl donor, but also a reaction medium. It was expected to increase the contact between the reactants in such a reaction system. However, the amount of formic acid consumed in the reaction was not as much as expected. Therefore, it is not feasible to use formic acid as an acyl donor and a reaction medium at the same time.

The esterification of KGM in a reverse micellar system was also studied using Lipase-OF as a biocatalyst. The composition and amount of reactants, including a substrate, an acyl donor and an enzyme, were the same as that in test No. 1. The reverse micellar system, in which the water-to-surfactant molar ratio (w_0) was 30, consisted of cyclohexane-Tween 80-H_2O and *n*-hexane-SDS (or sodium dodecyl sulphate)-H_2O, respectively. The consumed acyl donor in the process of the reaction was only 6.0% and 5.0%, respectively, thus indicating that the adopted reaction systems did not work very well.

A further investigation was made into the transesterification of KGM in another reversed micellar system. This system consisted of isooctane-AOT [or bis (2-ethylhexyl) sulfosuccinate]-buffer solution (pH 6.8) and contained much water due to its high water-to-surfactant molar ratio (w_0 = 50), so that it should dissolve the substrate (180 mg) better. In this transesterification reaction 500 mg of methyl stearate and 40 mg of Lipozyme Tl 100 L were used as an acyl donor and as a biocatalyst, respectively. The obtained product was characterized by FTIR as shown in Fig. 2. For the sake of convenient comparison and analysis, the infrared spectra of the original KGM and the enzymatically degraded KGM used as a substrate were also recorded and showed in Fig. 2.

It can be seen from Fig. 2 that there are obvious differences in the FTIR spectra of these three samples. Compared with the spectra of original KGM and degraded KGM, the broad strentching band of —OH groups on the backbone of a polysaccharide around 3200 ~ 3600 cm^{-1} becomes more narrow and is shifted to higher wave-number in the spectrum of the esterified KGM, indicating that the assocoation of intra-molecular hydrogen bonds is weakened in the esterified KGM. Accordingly, in the spectrum of the esterified KGM appear two strong absorption bands at 2918 cm^{-1} and 2850 cm^{-1}, which are respectively contributable to the asymmetric and symmetric stretching vibrations of $-CH_2-$ in an aliphatic hydrocarbon chain, and a weak absorption band at 2960 cm^{-1} assigned to the asymmetric stretching

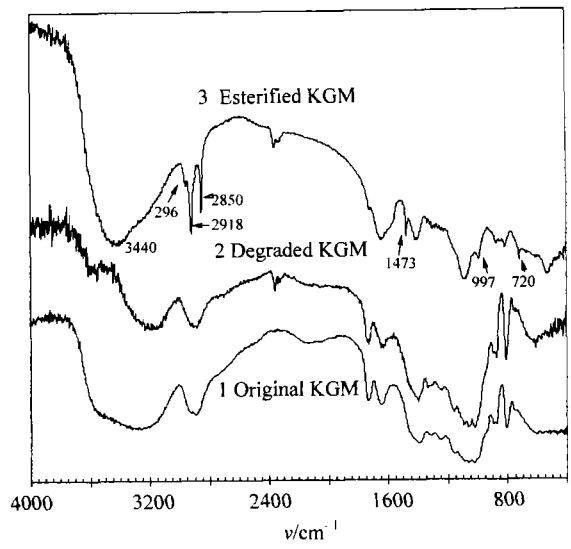

Fig. 2 FTIR spectra of the original KGM, the degradated KGM and the esterified KGM prepared by the transesterification reaction in a reverse micellar system

vibration of $-CH_3$ in an aliphatic hydrocarbon chain[18]. They seem to subtitute for the broad band around 2900 cm^{-1} corresponding to the stretching of methyl group of $-COCH_3$ in the original KGM[19]. Moreover, two new absorption bands appear at 1473 cm^{-1} and 720 cm^{-1}, which are respectively assigned to the in-plane and out-of-plane bending vibrations of $-(CH_2)_n-$ ($n \geqslant 4$)[20]. These analysis results imply that on the backbone of the esterified KGM exist long aliphatic hydrocarbon chains due to the transesterification reaction between KGM and methyl stearate. Thus, the designed transesterification reaction conducted in the reverse micellar system can work effectively. The appearance of another new band at 997 cm^{-1} could not be identified at this point.

The preliminary study suggests that the esterified derivatives of KGM can be prepared by lipase-catalyzed reactions in non-aqueous systems as expected. It is only an initial attempt to prepare such derivatives of a polysaccharide KGM through an environmentally benign enzymatic reaction. There is still much to do on the effects of each parameter upon the enzymatic reactions and the optimization of reaction conditions.

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References:

[1] Nishinari K, Williams P A, Phillips G O. *Food Hydrocolloids*, 1992, 6(2):199.

[2] Davé V, McCarthy S P. *J Environ Polym Degrad*, 1997, 5

(4): 237.
[3] Maeda M, Shimahara H, Sugiyama N. *Agric Biol Chem*, 1980, 44(2): 245.
[4] Jia C, Chen S, Mo W, et al. *Chinese Biochemical Journal*, 1988, 4(5): 407.
[5] FMC Corp., Nutricol® Konjac general Technology Bulletin, Food Ingredients Division, 1994.
[6] Aoyama Y, Matsumoto H, Tsuda T. *Agrci Biol Chem*, 1988, 52(11): 2811.
[7] Mizutani T, Mitsuoka T. *Cancer Lett*, 1982, 17(1): 27.
[8] Mizutani T, Mitsuoka T. *Cancer Lett*, 1983, 19(1): 1.
[9] Luo D, Li Y. *Chinese J Oncology*, 1992, 14(1): 48.
[10] Wu J, Peng S. *Acta Nutrimenta Sinica*, 1993, 15(2): 130.
[11] Ohya Y, Ihara K, Murata J, et al. // Vincenzini P, ed. *Intelligent Materials and Systems*. ©Techna Sri, 1995: 273.
[12] Gan X. CN 99116531.4.
[13] Tian B, Dong C. *L Chen*, 1998, 67(6): 1035.
[14] Feng P, Chen X, Yang D, et al. *Chemical Research and Application*, 1995, 7(3): 326.
[15] Li G, Qi L// IUPAC World Polymer Congress 2002, 39th International Symposium on Macromolecules, July 7 - 12, 2002, Beijing, China. Preprints (Part 2): 1064.
[16] Qi L, Li G, Zong M. *Acta Polymerica Sinica*, *in press*.
[17] Sun J, Wu B. *Nonaqeous Titration*. Beijing: Science Press, 1983.
[18] Yin J, Mo Z. *Modern physics for polymers*. Beijing: Science Press, 2001: 886.
[19] Maekaji K. *Agric Biol Chem*, 1974, 38(2): 315.
[20] Wang K, Luo C, Zhou X. *Modern Instrument Analysis for Polymers* (The 2nd Version), Beijing: Tsinghua University Press, 2000:32.

非水相体系中的脂肪酶催化反应制备酯化葡甘聚糖的初步研究

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摘 要:研究了在各种非水相反应体系中以不同的脂肪酶为催化剂的魔芋葡甘聚糖(KGM)的酶催化酯化和转酯化反应,以探索制备酯化 KGM 衍生物的可行的、环境友好的新途径。初步的实验结果表明:通过非水相反应体系中的脂肪酶催化反应能够制得 KGM 的酯化衍生物;在适宜的条件下,脂肪酶 Lipase-OF 和 Lipozyme TI 100L 是制备酯化 KGM 的有效的生物催化剂。

关键词:葡甘聚糖; 脂肪酶; 生物催化剂; 非水相体系; 酯化
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