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Enzymatic esterification between *n*-alcohol homologs and *n*-caprylic acid in non-aqueous medium under microwave irradiation

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Abstract

The enzymatic esterification between *n*-alcohol homologs and *n*-caprylic acid catalyzed by lipozyme RM IM (LRI) in microwave field was investigated. Some interesting findings were obtained. The optimum reaction temperature slightly shifted from that in enzymatic esterification by conventional heating. *n*-Alcohol homologs used in this experiment showed substrate specificity in terms of the odd and even carbon numbers. THF expressed abnormal solvent effect. Whereas in the contrastive enzymatic esterification by conventional heating, the above mentioned substrate specificity and solvent effect were not observed. All the above phenomena could be explained by both thermal and non-thermal effect of microwave on enzyme and substrates. Further investigation revealed that microwave irradiation reduced the apparent activation energy of the enzymatic reaction according to *Arrhenius* equation, which is considered as one of the causes increasing initial reaction rate.

Keywords: Microwave irradiation; Enzymatic esterification; Solvent effect; Apparent activation energy

1. Introduction

Since last decade, researchers have attempted to use microwave to improve enzymatic reactions in non-aqueous medium [1–3], which has been well reviewed recently [4]. As indicated by Parker et al. [2], the enzymatic transesterification rate between butanol and ethyl butyrate was enhanced 2–3 folds by microwave irradiation over conventional heating. Ipsita and Gupta [3] also observed that microwave irradiation increased the initial reaction rates by 2.1–4.7 times at all levels of (trans-) esterification and discussed the potential non-thermal effect of microwave on enzymatic reactions. It is generally believed that microwave irradiation (MI) has obvious advantages over conventional heating (CH) in some enzymatic reactions, in which reaction rate, yield and selectivity such as stereo-selectivity or region-selectivity are obviously influenced [1–6]. It is worth noting that most previous work focused on the microwave heating technique and the reaction parameter optimization under MI, while less attention has been paid on the non-thermal microwave effect on enzymatic reactions and its origin. Therefore, to dig out the special effects or phenomena in microwave-assisted enzymatic reaction, which do not exist in enzymatic reaction by conventional heating, will be beneficial to illustrating the mechanism of microwave-assisted enzymatic reaction.

We recently reported the lipase-catalyzed esterification between pentanol isomers and *n*-caprylic acid under discontinuous microwave irradiation [7]. The results showed that microwave irradiation increased the reaction rates by 2.5–4.5 times. In the present work, we further investigated microwave effect on enzymatic esterification of C_2–C_{10} *n*-alcohol homologs and *n*-caprylic acid in non-aqueous medium. The reactions were performed in a homemade microwave reactor characterized by continuous and steady irradiation with temperature control ($\pm 1\,^{\circ}C$).

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2. Experimental

2.1. Chemicals

Lipozyme RM IM (LRI), a lipase from *Mucor miehei* immobilized on an anionic resin was a gift sample from Novo Nordisk Co. Isolated *n*-alcohol homologs (C_4 to C_{10}) were of reagent grade, other reagents including ethanol and *n*-propanol were of analytical grade. All reagents were dried with 4 Å molecular sieves before use. The deionized water was used, if any, throughout the all experiment.

2.2. Microwave enzyme reactor

The microwave enzyme reactor was configured with a glass reactor, a microwave-generator and a temperature control system. The reaction mixture was placed into a glass cylinder reactor with cooling jacket. The enzymatic esterification was performed in a modified microwave oven (NN-S552, National Co., 2450 MHz) equipped with a magnetic stirring apparatus and a sampling hole. The reaction temperature was controlled with a microwave-shielding thermo sensor, which is connected with an electromagnetic feedback loop to monitor the flow of cooling medium.

2.3. Analysis

Conversion of caprylic acid was defined as the percentage of consumed caprylic acid and measured by titration. The initial reaction rate of the esterification was defined as the mmoles of ester produced from one gram of acid per minute in the initial phase of reaction, and was calculated based on the amount of consumed *n*-caprylic acid in the first 5–10 min, during which the conversion of caprylic acid was exactly in direct proportion to the reaction time.

2.4. Microwave irradiation-enzyme coupling catalysis (MIECC)

2.4.1. Solvent-free esterification

In a 20 ml glass reactor, caprylic acid (25 mmol), *n*-alcohol (25 mmol) and water (1%, w/w) were homogenized under the desired temperature. Then the reactor was placed into the modified microwave oven. The reaction started once the enzyme LRI (50 mg) was added and well mixed with the reaction mixture under magnetic stirring (400 rpm) accompanied by continuous microwave irradiation (200 W).

2.4.2. Esterification in solvent phase

In a 20 ml glass reactor, caprylic acid (5 mmol), *n*-alcohol (5 mmol), organic solvent (10 ml) and water (1%, w/w) were well mixed. All other operating conditions were the same as that in solvent-free esterification except the dosage of enzyme LRI was 10 mg.

2.5. Enzymatic esterification by conventional heating

In a 20 ml glass reactor, caprylic acid (25 mmol), *n*-alcohol (25 mmol), organic solvent (10 ml) if any, and water (1%, w/w) were homogenized under the desired temperature. The reaction started when the enzyme LRI (50 mg) was added and well mixed with the reaction mixture under magnetic stirring (400 rpm), the temperature of the reaction was maintained constant to the desired using a water bath.

3. Results and discussion

3.1. The optimum temperature for enzymatic esterification assisted by microwave

The initial reaction rates at different temperatures are shown in Fig. 1. The initial reaction rate under CH increased with increasing reaction temperature from 40 to 50 °C. The similar trend was observed under MIECC, where the highest initial reaction rate appeared at 55 °C. The reason that at each tested temperature the initial reaction rate under MIECC is higher than that under CH may be explained due to both thermal and non-thermal effect of microwave irradiation as follows. First of all, under MIECC, the polar molecules (the alcohol and the acid) collide with each other because of thermal effect and microwave effect. Therefore, the molecule collision under MIECC has extra driving force compared to that under CH, which results higher rate under MIECC as long as the enzyme is not deactivated by microwave. Since all MIECC reactions were applied under 200 w with the same amount and the same kind of reaction mixture, the contribution of microwave to each reaction are supposed to be the same, which is implied by the parallel curves in Fig. 1. One can assume that the bias in *Y*-axis of Fig. 1 is the contribution of microwave to reaction rate. Secondly, one of the non-thermal effects is that microwave energy can also modulate the configuration of enzyme molecules by accelerating the molecular rotation and electron spin oscillation of the polar parts of enzyme, which can provide more chance to make the substrates fit to the enzyme in unit of time.

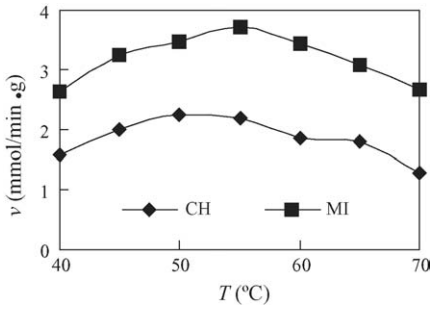

Fig. 1. Initial reaction rate of solvent-free enzymatic esterification (♦: CH, ■: MI).