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Kinetic Study of Deacetylation and Acetyl Migration of Peracetylated 1-Methyl α,β-D-Glucopyranosides by *Candida* Lipase-Catalyzed Hydrolysis

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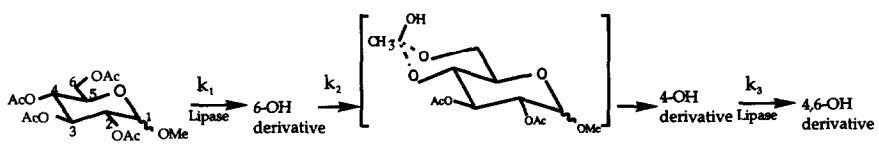
Abstract : Peracetylated 1-methyl α,β-D-glucopyranosides (**1** and **2**) were hydrolyzed by *Candida* lipase and 6-OH, 4-OH and 4,6-OH derivatives were obtained. A kinetic study revealed that the reaction was a consecutive irreversible three-stage reaction including one acetyl migration and two enzymatic deacetylation reactions. The three rate constants were measured and theoretical profiles for predicting the amount of products and substrates against reaction time were determined.

Lipases have been widely used for the regioselective deacylation of polyhydroxyl molecules including carbohydrates. According to the previous reports,[1-4] *Candida* lipase preferentially cleaves the 6-acyl group of peracetylated 1-methyl α-D-glucopyranoside (**1**). However, the major 6-OH derivative was usually accompanied by the 4-OH and 4,6-OH derivatives as minor products and the ratio of the minor products was found to increase in proportion to reaction time. In order to study the mechanism of this reaction, the reaction solution was carefully analyzed by HPLC at different reaction time intervals and the proposed mechanism of formation of products may be illustrated as follows:



C-4 ------> C-6 acetyl migration
glucose derivative intermediate

First, the lipase cleaved the 6-acetyl group to form the 6-OH derivative, then the 4-OH derivative was generated from 6-OH derivative by 4 ---> 6 acetyl migration[5,6] through a six-member ring transition[7] and finally the lipase further hydrolyzed the new-born 6-ester to provide the 4,6-OH derivative. *Candida* lipase does not readily hydrolyze secondary esters and yet the 4,6-OH derivative is the final product in the reaction. Because the hydrolytic reactions were conducted in aqueous solution, the first and third reactions were considered as irreversible hydrolysis. In addition, the acetyl migration in the second reaction may also be irreversible due to the favorable secondary to primary alcohol acetyl migration and the subsequent hydrolysis of the product 4-OH derivative. Therefore, the entire reaction can be considered as a first-order and consecutive irreversible three-stage reaction.

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \xrightarrow{k_3} D$$

The rate constants (k_1, k_2 and k_3) of three consecutive reactions could be measured and calculated.[8] Based on the three rate constants, the quantitative prediction of the substrate and three products at any reaction time could be illustrated. In the typical experiments,[9] compounds **1** and **2** were hydrolyzed by *Candida* lipase and three rate constants (k_1, k_2 and k_3) were calculated, respectively. The quantitative profiles of substrates and products against reaction time were generated by computer and shown in Fig 1. In the case of **1**, k_1 is much larger than k_2, and the 6-OH derivative accumulated; while in the case of **2**, k_1 is only slightly larger than k_2, and the 6-OH derivative hardly accumulated and was further converted into the 4-OH and 4,6-OH derivatives. The configuration of 1-OCH_3 in **1** and **2** had a great influence in the hydrolytic rate of 6-acetyl group by *Candida* lipase. These results reveal that (a) the 6-OH derivative from **1** could be obtained in high yield in the short time reaction, and (b) reactions conditions which can prevent or slow down the rate of acetyl migration provide the 6-OH derivatives from **2** in high yield.

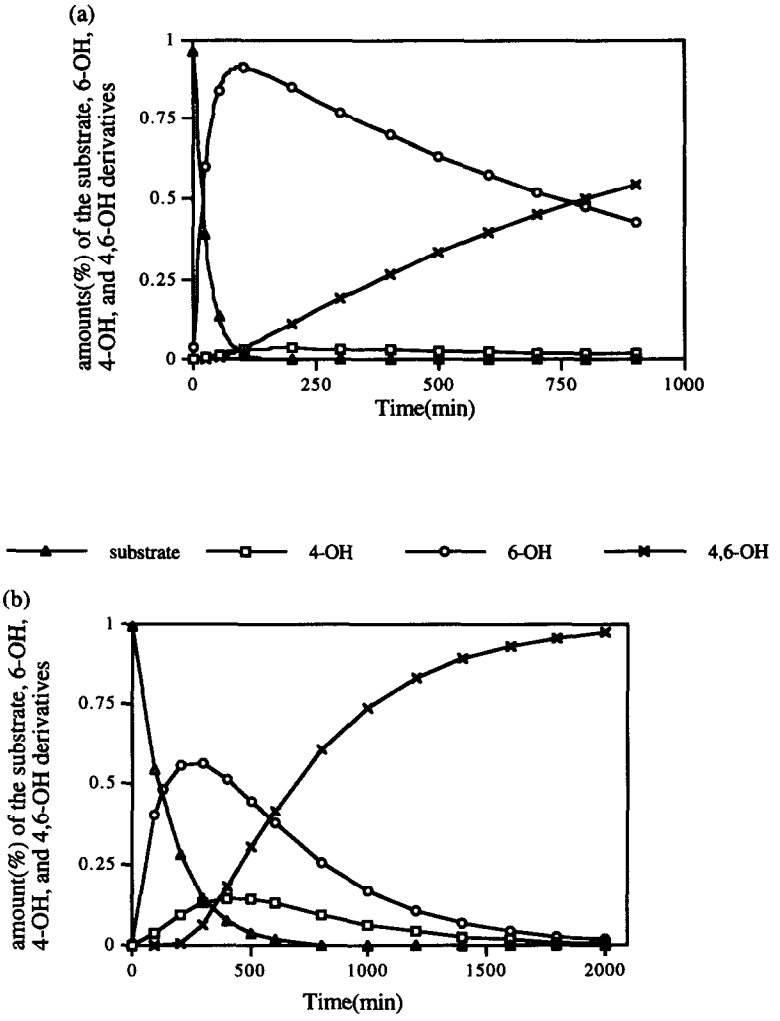


Fig. 1 Plots of amount percent of substrates and products as a function of reaction time. (a) For the case of **1**, the curves were generated using the rate constants k_1 = 0.03896, k_2 = 0.00096, k_3 = 0.02206 (min^{-1}) (b) For the case of **2**, the curves were generated using the rate constants k_1 = 0.00640, k_2 = 0.00218, k_3 = 0.00751(min^{-1}).

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   The three rate constants in the first-order and consecutive irreversible three-stage reaction could be calculated from the following equations :

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \xrightarrow{k_3} D$$

$[A] = [A]_o \cdot e^{-k_1 t}$ ------ (1)

$[B] = [A]_o\left[e^{-k_1 t} \cdot k_1/(k_2-k_1) + e^{-k_2 t} \cdot k_1/(k_1-k_2)\right]$ ----- (2)

$[C] = [A]_o\left[e^{-k_1 t} \cdot k_2k_3/(k_2-k_1)(k_3-k_1) + e^{-k_2 t} \cdot k_1k_2/(k_1-k_2)(k_3-k_2) + e^{-k_3 t} \cdot k_1k_2/(k_1-k_3)(k_2-k_3)\right]$ ------ (3)

$[D] = [A]_o\left[1 - e^{-k_1 t} \cdot k_2k_3/(k_2-k_1)(k_3-k_1) - e^{-k_2 t} \cdot k_1k_3/(k_1-k_2)(k_3-k_2) - e^{-k_3 t} \cdot k_1k_2/(k_1-k_3)(k_2-k_3)\right]$ ------ (4)

9. To a solution of 1.5 g (1.47 mmol) of the substrate (**1** or **2**) in 50 mL of phosphate buffer (pH 7.0, 0.1 M containing 0.2 M NaCl, 3 mM $CaCl_2$ and 5 mL of acetone) was added *Candida* lipase (3.0 g). The amounts of substrate and products at different reaction intervals were determined by HPLC with calibration. The HPLC with a C_{18} column (10 μm particle size, 300 x 4.6 mm) was eluted with a gradient of 10-10-49 % CH_3CN in double distilled water at 0-5-30 min at a 1 mL/min of flow rate and with UV 214 nm monitoring. The elution times are 8.12 min for 4,6-OH, 20.00 min for 4-OH, 23.11 min for 6-OH, and 28.93 min for **1** and 8.99 min for 4,6-OH, 19.35 min for 4-OH, 19.99 min for 6-OH, and 28.47 min for **2**. The data were applied to calculate the rate constants (k_1, k_2 and k_3).

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