Journal of Molecular Catalysis B: Enzymatic 63 (2010) 17–22

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Study on acyl migration kinetics of partial glycerides: Dependence on temperature and water activity

Wei Li, Wei Du*, Qiang Li, Ting Sun, Dehua Liu*

Department of Chemical Engineering, Tsinghua University, Beijing 100084, PR China

ARTICLE INFO

Article history:
Received 19 June 2009
Received in revised form
29 November 2009
Accepted 30 November 2009
Available online 2 December 2009

Keywords:
Kinetics
Acyl migration
Partial glycerides
Water activity

ABSTRACT

Acyl migration phenomenon was often observed during 1,3-positional specificity lipase-catalyzed reactions from triglycerides and partial glycerides, including acyl migration of 1,2-diglyceride (1,2-DG) to 1,3-diglyceride (1,3-DG) and 2-monoglyceride (2-MG) to 1-monoglyceride (1-MG). However, the acyl migration mechanism and kinetics were seldom studied despite of numerous researches on process optimization of 1,3-positional specificity lipase-catalyzed reaction. In this paper, the influence of related factors on acyl migration process as well as their influencing mechanism was further studied. It was found that temperature and water activity were two crucial factors that would influence acyl migration kinetics. Determination of the kinetic parameters under different temperatures revealed that the acyl migration reaction rates were greatly promoted by the increasing of temperature. The acyl migration rates of 1,2-diglyceride and 2-monoglyceride were quite different from each other, which was found to be due to the different activation energies. Further study of how would water influence the acyl migration process showed that water activity rather than water content was a key factor that influenced acyl migration and the acyl migration rate would decrease with the increase of water activity. It was further revealed that water activity influenced the charge dispersion of the transition state, which ultimately influenced the reaction activation energy and then influenced the acyl migration rate.

1. Introduction

Biodiesel, which is defined as methyl esters derived from plant oils and animal fats, has attracted numerous attention as a clean and renewable energy, since it is one of the possible solutions to solve the world wide problem of energy crisis and environmental protection [1–3]. Conventional chemical ways for biodiesel production are well developed but has the disadvantage of high-energy requirements, difficulties in the recovery of catalyst and glycerol, as well as environmental pollution. On the contract, enzymatic biodiesel production has the merits of mild reaction conditions, low raw material requirements and easy product recovery compared to chemical ways [4,5]. For long-term development, edible oils such as vegetable oils and animal fats cannot be used preferably as the feedstocks for biodiesel production. Enzymatic approaches will have great prospect since they can be applied to biodiesel production based on relatively poor quality oil feedstocks [3–5].

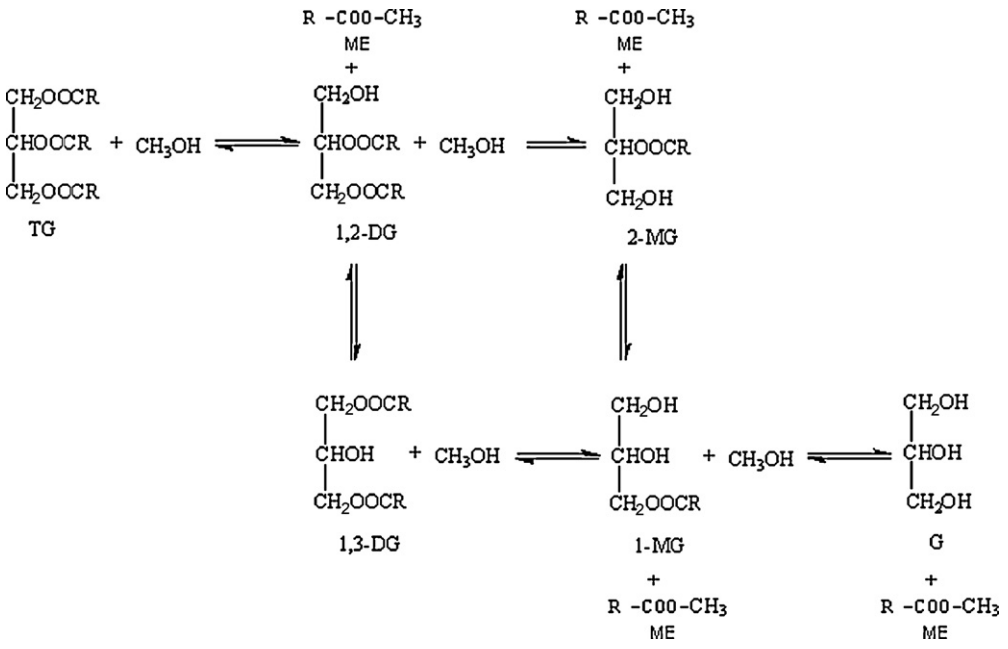
Some lipases and whole cell catalysts for biodiesel production were reported to have 1,3-specificity towards substrate triglycerides [6–9]. During 1,3-positional specificity lipase-catalyzed biodiesel production from triglyceride, partial glycerides of 1,2-diglyceride and 2-monoglyceride would accumulate in the reaction medium and the theoretical methyl esters yield was only 67%. While in the process of some 1,3-positional specificity lipase-catalyzed biodiesel production, the reported biodiesel yield could research over 80% [6–9]. In our previous study of *Rhizopus oryzae* (with intracellular 1,3-positional specificity lipase)-mediated methanolysis of triolein, it was revealed that higher practical methyl ester yield over theoretical yield was partly due to acyl migration of 1,2-DG to 1,3-DG and 2-MG to 1-MG (Scheme 1), which further progressed the methanolysis reaction. So obviously acyl migration was important to the 1,3-positional specificity lipase-catalyzed biodiesel production, since it is vital to the biodiesel yield.

Acyl migration is also an important phenomenon during 1,3-positional specificity lipase-catalyzed production of partial glycerides, which are useful in a broad range of food, cosmetic and pharmaceutical applications, especially in the production of partial glycerides in a particular isomeric form [10,11]. Acyl migration during this process was also crucial to the ratio and purity of different partial glyceride isomers such as 1,3-DG, 1,2-DG, 1-MG and 2-MG.

It was found that acyl migration could take place independent of enzyme catalysis [14–16]. That means, acyl migration between 2-

* Corresponding authors. Tel.: +86 10 62772130; fax: +86 10 62772130.
E-mail addresses: duwei@tsinghua.edu.cn (W. Du), dhliu@tsinghua.edu.cn (D. Liu).

1381-1177/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2009.11.012

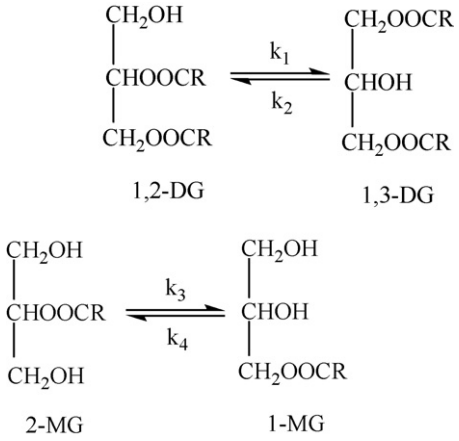


Scheme 1. 1,3-Positional specificity lipase-catalyzed methanolysis with acyl migration.

MG and 1-MG as well as acyl migration between 1,2-DG and 1,3-DG would occur with the absence of enzyme. And the acyl migration was found to be first-order reversible reaction (Scheme 2).

Although acyl migration was always observed during the process of 1,3-positional specificity enzyme-mediated process, few reports were found on the study of mechanism and related kinetic of acyl migration process alone. Murgia et al. [12] studied the extent of acyl migration and hydrolysis phenomena in glycerol monooleate/water-based systems and how they were influenced by phase transition qualitatively. Laszlo et al. [13] have done some research on the modeling of acyl migration kinetic of 1,2-diacylglycerols. Fureby et al. [14], Vikbjerg et al. [15] and Oda et al. [16] have done some research on the influence of reaction parameters on acyl migration. However, their research was carried out in the process of enzyme-catalyzed reaction. During these processes, some reaction parameters might have effect on both enzyme-catalyzed reaction and acyl migration. Therefore, it was hard to decide how these factors would influence acyl migration alone exactly.

Temperature and water activity were two crucial factors that would influence enzyme-mediated process including acyl migration. In this paper, the effect of temperature and water activity on acyl migration process and kinetics alone was further studied and the related mechanism was explored for the first time.



Scheme 2. Acyl migration between partial glycerides.

2. Materials and methods

2.1. Materials

Triolein used as substrate was obtained locally. Palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and heptadecanoic acid as well as their methyl esters as GC standards and 1-olein, 2-olein, 1,2-diolein, 1,3-diolein and triolein as HPLC standards were purchased from Sigma and were chromatographically pure. Other chemicals were obtained commercially and were of analytical grade. *R. oryzae* IFO4697 whole cell immobilized within biomass support particles was cultivated and prepared in our laboratory as described in our previous study [8].

2.2. Methanolysis of triolein

The methanolysis reactions were carried out in 50 ml screw-cap bottles at 35 °C on a reciprocal shaker (130 oscillations/min). The compositions of the reaction mixtures were as follows: 10 g triolein, 12 g tert-butanol, 1.46 g methanol, and 0.3 g 0.1 mmol/l phosphate buffer (pH 6.8). 50 μl samples were taken from the reaction mixture at specified time, centrifuged to obtain the upper layer and analyzed by GC and HPLC. Each condition was done in duplicates.

The methyl esters contained in the reaction mixture were analyzed using a GC-14B gas chromatography (Shimadzu, Kyoto, Japan) equipped with a CP-FFAP CB capillary column (25 m × 0.32 mm × 0.30 μm) supplied by Agilent. The aforementioned mixture (5 μl) and 300 μl of 1.4 mmol/l heptadecanoic acid methyl ester (hexane as the solvent) which is served as the internal standard and ethanol (300 μl) were mixed thoroughly. The column temperature was kept at 180 °C for 0.5 min, heated to 250 °C at 10 °C/min, and then maintained for 6 min. The temperatures of the injector and detector were set at 245 °C and 250 °C, respectively.

Table 1
Gradient elution program.

Time (min)	Acetonitrile–acetic acid (99.85:0.15) (V/V, %)	Dichloromethane (V/V, %)
0	100	0
4	100	0
12	90	10
25	90	10
30	70	30
35	70	30
45	20	80
55	20	80
60	100	0
65	100	0

Note: Analysis is performed as a constant flow rate of 1.5 ml min^{-1}.

2.3. Acyl migration study

1 ml reaction mixture of the aforementioned methanolysis reactions was taken at 6 h' reaction and used for acyl migration study. The acyl migration reactions were carried out in 5 ml sealed tubes at specified temperature in a thermostatic container. Samples of 50 μl were taken from the reaction mixture at specified time and analyzed by HPLC. Each condition was done in duplicates.

2.4. Water activity control

The substrates were first dried using 3 Å molecular sieves, and then pre-equilibrated for at least 3 d in separate sealed containers enclosed with saturated salt solutions or solid adsorbent to establish fixed water activities. The corresponding water contents of different water activities were measured by Karl Fischer titration. Before the start of the acyl migration reaction, the substrates were adjusted to specified water activities by adding corresponding water content into the substrates, and then put into the pre-equilibrated sealed containers to maintain the activities [17]. 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 $MgCl_2$ (a_w: 0.328), $NaNO_3$ (a_w: 0.743), K_2SO_4 (a_w: 0.973) [17].

2.5. Analysis of the samples

The glyceride and partial glycerides in the reaction mixture were analyzed by a Shimadzu 20A HPLC system (Shimadzu Corp., Kyoto, Japan) with an ELSD-LTII low temperature-evaporative light scattering detector. 2 μl sample and 1 ml acetone were precisely measured and mixed thoroughly. The aforementioned mixture (20 μl) was injected. The stationary and mobile phases were an C_{18} column (5 μm, 250 mm × 4.6 mm) (Dikma Technology, PLATISIL ODS, China) and a gradient elution program by acetonitrile and dichloromethane at 1.5 ml min^{-1} (Table 1) respectively. The column temperature was controlled at 40 °C. The drift pipe temperature was controlled at 70 °C, and the nitrogen pressure was controlled at 320 kPa.

3. Results and discussion

3.1. Effect of temperature on acyl migration

During the process of *Rhizopus oryzae* (*R. oryzae*)-mediated methanolysis for methyl ester synthesis, 1,2-DG and 2-MG were found to accumulate in the reaction mixture at the initial reaction period, which was mainly due to the positional specificity of the lipase [16]. It was found that acyl migration between 2-MG and 1-MG as well as acyl migration between 1,2-DG and 1,3-DG would take place with the absence of the enzyme [14–16]. Here the effect of temperature on acyl migration process alone was first investigated through kinetic study of acyl migration under various temperatures.

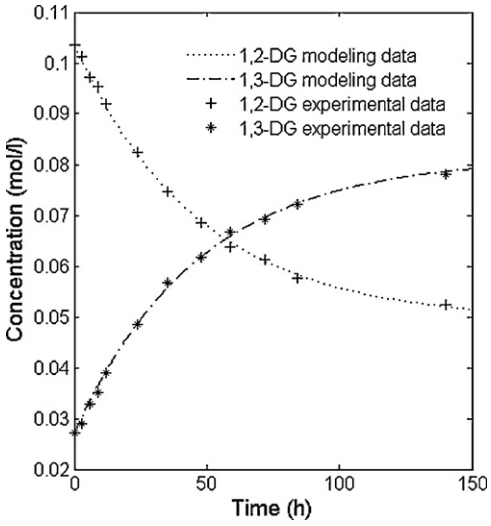


Fig. 1. Acyl migration of 1,2-DG to 1,3-DG at 30 °C.

The reaction of *R. oryzae*-mediated methanolysis of triolein was stopped at 6 h to obtain abundant 2-MG and 1,2(2,3)-DG. Then the resultant mixture was kept at different temperatures and the content of 1,2-DG, 1,3-DG, 2-MG and 1-MG was analyzed with time proceeding. Firstly the acyl migration kinetics of 1,2-DG to 1,3-DG and 2-MG to 1-MG were studied and modeled under a first-order reaction reversible scheme as Scheme 2 showed. The differential equations characterizing the acyl migration reactions were as follows:

$$\frac{d[1,2-\mathrm{DG}]}{dt} = -k_1[1,2-\mathrm{DG}] + k_2[1,3-\mathrm{DG}]$$
$$\frac{d[1,3-\mathrm{DG}]}{dt} = -\frac{d[1,2-\mathrm{DG}]}{dt} \quad (1)$$

$$\frac{d[2-\mathrm{MG}]}{dt} = k_3[2-\mathrm{MG}] - k_4[1-\mathrm{MG}]$$
$$\frac{d[1-\mathrm{MG}]}{dt} = -\frac{d[2-\mathrm{MG}]}{dt} \quad (2)$$

where [1,2-DG], [1,3-DG], [2-MG] and [1-MG] were the molar concentrations of 1,2-DG, 1,3-DG, 2-MG, and 1-MG, respectively.

The concentration change of each component was obtained experimentally and the non-linear curve fitting software Matlab was used for fitting the system of differential Eqs. (1) and (2) into the experimental data. Comparison between modeled and experimental data under 30 °C was presented in Figs. 1 and 2. It could be seen that a good agreement was obtained. During the reaction process, 1,2-DG and 2-MG decreased gradually, while 1,3-DG and 1-MG increased gradually. Equilibrium between 1,2-DG and 1,3-DG, as well as between 2-MG and 1-MG was reached after certain time.

Comparison between acyl migration of DG and MG indicated that the rates of migration of the 2-position fatty acid moiety in 2-MG were much higher than those in 1,2-DG, which was understandable because 2-MG apparently had two acyl acceptor, while 1,2-DG had only one, as could be seen in Scheme 2. Therefore, the attacking chances of acceptor were twofold in 2-MG than that in 1,2-DG. However, due to this mechanism, the difference of acyl migration rates could only be twofold of 2-MG to 1,2-DG. While the experimental result of acyl migration rate constant of 2-MG under

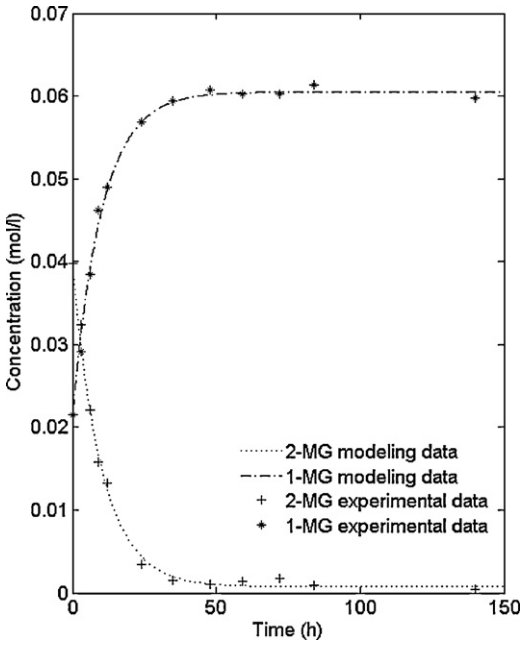


Fig. 2. Acyl migration of 2-MG to 1-MG at 30 °C.

30 °C was nearly 7.4-fold of that of 1,2-DG. Therefore, the activation energies of the acyl migration reaction of 2-MG and 1,2-DG must be quite different from each other.

Acyl migration process was further modeled under various temperatures, and the rate constants and half-life values were calculated and listed in Table 2. Similar results and good agreements of modeled data with experimental data were also obtained (data not shown).

From Table 2, it could be seen clearly that the acyl migration constants of both 1,2-DG and 2-MG increased greatly along with the increasing of temperature. The half-life values of 1,2-DG and 2-MG under 30 °C were 132.53 h and 7.20 h, while they were only 13.80 h and 1.20 h under 55 °C. The half-life values of 2-MG were always much shorter than that of 1,2-DG under a certain temperature. The equilibrium state of both DG and MG seemed to be constant under different temperatures. The mole ratios of 1,2-DG and 2-MG at equilibrium were found to be 0.38 and 0.05, respectively.

Based on data in Table 2, the Arrhenius plots of acyl migration reaction were showed in Fig. 3. From the Arrhenius plots of k_1 and k_3 values, the apparent activation energies were evaluated to be 73.8 and 59.4 kJ/mol, respectively.

Difference in activation energy between acyl migration of 1,2-DG and that of 2-MG also illustrated their difference in acyl migration rate constants. Therefore, higher apparent activation energy of 1,2-DG and fewer acyl acceptors compared to that of 2-MG together contributed to the lower acyl migration rate of 1,2-DG to 1,3-DG than that of 2-MG to 1-MG. Yang [18] also reported the effect of temperature on acyl migration and through temperature programming, the suppression of acyl migration could be achieved without loss of reaction yield.

Table 2
Comparison of first-order reaction constants and half-life values for acyl migration of DG and MG under various temperatures.

Rate constants and half-life value	Temperature			
	55 °C	45 °C	37 °C	30 °C
k_1 (h^{-1})	0.128	0.0451	0.0252	0.0132
k_1/k_2	1.65	1.66	1.64	1.65
k_3 (h^{-1})	0.581	0.274	0.152	0.0975
k_3/k_4	20.8	20.6	20.6	20.7
$t_{1/2DG}$ (h)[a]	13.8	36.0	76.3	132
$t_{1/2MG}$ (h)[a]	1.20	2.58	4.70	7.20

[a] $t_{1/2DG} = (k_1 + k_2)^{-1} \ln 2$, $t_{1/2MG} = (k_3 + k_4)^{-1} \ln 2$.

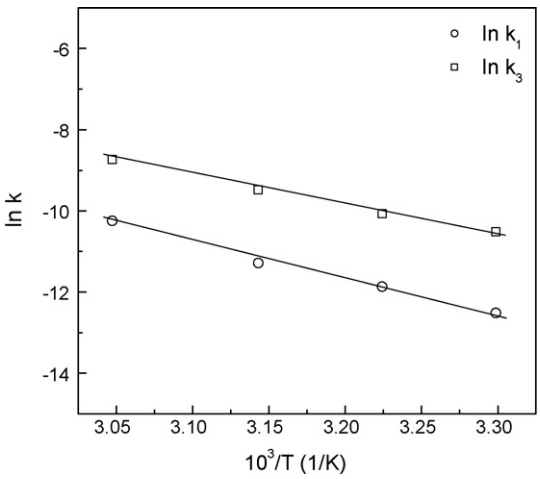


Fig. 3. Arrhenius plots of rate constants.

Table 3
Comparison of first-order reaction constants and half-life values for acyl migration of DG and MG under various water activities.

Rate constant and half-life value	Water activity			
	<0.01	0.328	0.743	0.973
k_1 (h^{-1})	0.0226	0.0194	0.0151	0.0146
k_1/k_2	1.66	1.65	1.64	1.65
k_3 (h^{-1})	0.112	0.0980	0.0820	0.0681
k_3/k_4	20.7	20.6	20.5	20.7
$t_{1/2DG}$ (h)[a]	78.2	94.6	136	176
$t_{1/2MG}$ (h)[a]	6.43	7.37	8.85	10.8

[a] $t_{1/2DG} = (k_1 + k_2)^{-1} \ln 2$, $t_{1/2MG} = (k_3 + k_4)^{-1} \ln 2$.

3.2. Effect of water activity on acyl migration

Besides temperature, water might be another important parameter influencing acyl migration. Some researchers have investigated the influence of water on acyl migration [14–16].

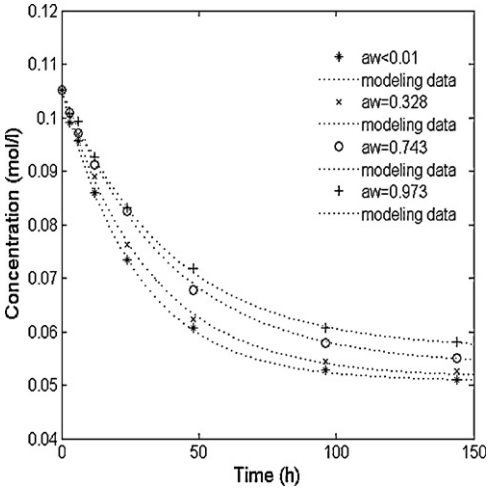


Fig. 4. Effect of water activity on acyl migration of 1,2-DG.

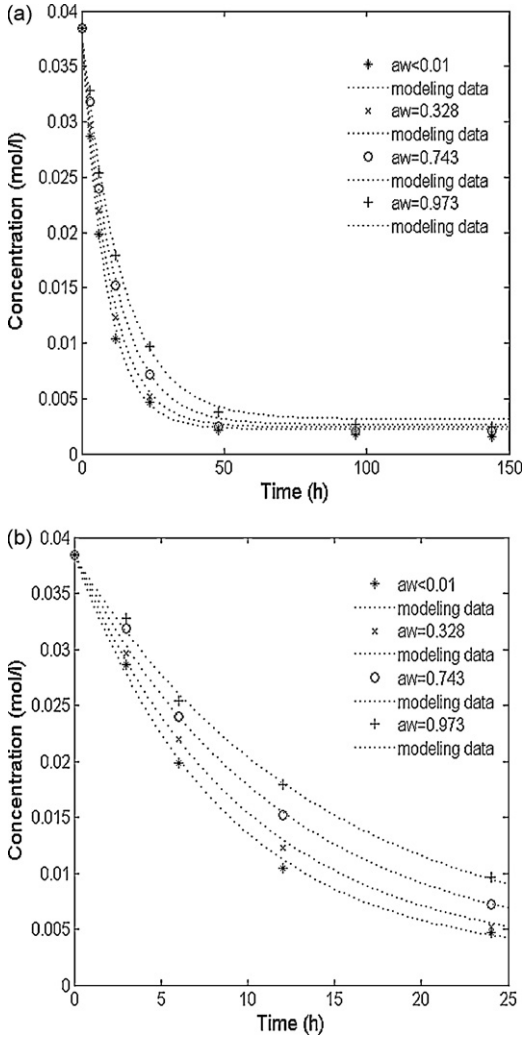


Fig. 5. Effect of water activity on acyl migration of 2-MG (graph b is a "focus" of the first 25 h of graph a).

However, their researches were carried out during the process of enzyme-catalyzed reaction. Just as the aforementioned effect of temperature, it was hard to tell the true effect of water on acyl migration alone. Results reported by Fureby et al. [14] showed that acyl migration favored a low water activity; while the result reported by Vikbjerg et al. [15] and Oda et al. [16] claimed a reverse conclusion. In this paper, the effect of water activity on acyl migration alone was first studied through modeling the acyl migration process and calculating the kinetic parameters of acyl migration under different water activities.

The reaction of *R. oryzae*-mediated methanolysis of triolein was stopped at 6 h to obtain abundant 2-MGs and 1,2(2,3)-DGs. Then the resultant mixture was kept at different water activities and the content of 1,2-DG, 1,3-DG, 2-MG as well as 1-MG was analyzed with time proceeding. The initial concentrations of 1,2-DG, 1,3-DG, 2-MG, and 1-MG in the reaction mixture were 0.105, 0.029, 0.038 and 0.024 mol/l, respectively. The change of 1,2-DG and 2-MG concentrations at different water activities were presented in Figs. 4 and 5 (Fig. 5b was the initial part of Fig. 5a). It was indicated by these figures that the acyl migration rates decreased with the increase of water activity.

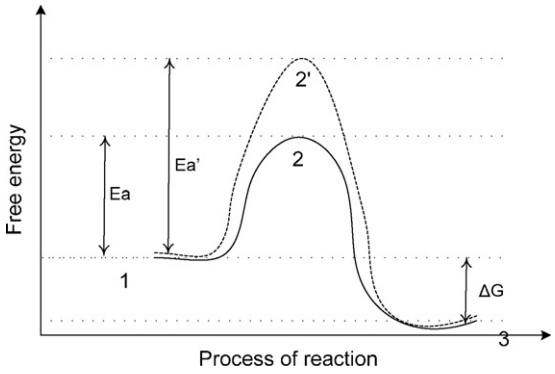


Fig. 7. Activation energy of acyl migration.

Further calculation of acyl migration rate constants and half-life values presented in Table 3 confirmed the negative effect of water on acyl migration. The half-life values of 1,2-DG and 2-MG were 175.93 h and 10.75 h at water activity of 0.973, and only 78.23 h and 6.43 h under dry condition. These results also indicated that the equilibrium states of both DG and MG were insensitive to water activity of the medium.

3.3. *Effect of water content on acyl migration*

In terms of water content, the above studied water activities were 0.22‰, 0.94‰, 1.84‰, 2.94‰, respectively, all of which in trace amount. In order to disclose how water influences the acyl migration reaction, water contents in larger amount were investigated further (from 1% to 30% of the initial triolein weight).

It was found that the acyl migration of both 1,2-DG and 2-MG did not vary much under such a wide range of water contents. Further calculation of acyl migration reaction constants and half-life values through kinetic study and modeling of first-order reversible reaction by Matlab confirmed this conclusion (data not shown). The results indicated that it was water activity rather than water content that influenced the acyl migration process.

Water activity (a_w) is a measurement of the energy status of the water in a system. It is defined as the vapor pressure of water divided by that of pure water at the same temperature. The highest water activity of the reaction system was 1, so further increasing the water content of the reaction system would not affect water activity of the system after it had reached 1.

The mechanism of water activity influencing acyl migration was further explored as follows. As presented in Fig. 6, acyl migration belonged to S_N2 nucleophilic substitution. For this kind of reaction, charge would disperse at transition state, as presented in Fig. 6, transition state 2. Fig. 7 explained the change of energy state during the reaction process. Along with the increasing of water activity in the reaction system, the polarity of the reaction medium increased, which went against the dispersion of charge in the transition

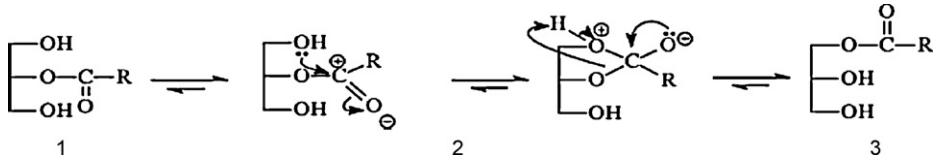


Fig. 6. Mechanism of acyl migration.

state. Accordingly, the energy state of the transition state increased (from 2 to 2′ in Fig. 7), which resulted in larger activation energy (Ea′ > Ea).

The acyl migration rate at certain temperature was thus decreased. While the energy state of reactant and product remain unchanged, ΔG of acyl migration reaction did not change, so the equilibrium of acyl migration was not affected by water activity, which was consistent with the result in Table 2.

4. Conclusions

Acyl migration belongs to a first-order revisable reaction mechanism and the kinetics of acyl migration was greatly influenced by temperature and water activity. The acyl migration rate increased dramatically with the increasing of temperature. A 1,2-diglyceride half life ($t_{1/2}$) of 1.380 h and 132.53 h, and a 2-monoglyceride half life ($t_{1/2}$) of 1.20 h and 7.20 h, were found at 55 and 30 °C, respectively. And great difference between acyl migration of 1,2-diglyceride with 2-monoglyceride was found in the reaction activation energy. Water activity was also found to be an influential factor that influenced acyl migration kinetics. Low water activity was favored for high acyl migration rate and it was revealed that water activity influenced the acyl migration rate through influencing the charge dispersion of the transition state, which ultimately influenced the reaction activation energy. Further increase of water content of the reaction mixture did not have significant effect on acyl migration rate, which illustrated water activity rather than water constant was a crucial factor that influenced acyl migration.

Acknowledgments

The authors express their thanks for the support from National Natural Science Foundation of China (20706034), National 863 High Tech Project (2006AA020203), 973 project (2007CB714302) as well as Tsinghua Fundamental Research Funding.

References

[1] H. Fukuda, S. Hama, S. Tamalampudi, H. Noda, Trends Biotechnol. 26 (2008) 668–673.
[2] W. Du, L. Wang, D.H. Liu, Green Chem. 9 (2007) 173–176.
[3] Y. Chisti, Trends Biotechnol. 26 (2008) 126–213.
[4] T.W. Tan, J.H. Xu, Y.J. Asano, Mol. Catal. B: Enz. 56 (2009) 77.
[5] W. Du, W. Li, T. Sun, X. Chen, D.H. Liu, Appl. Microbiol. Biotechnol. 79 (2008) 331–337.
[6] K. Ban, M. Kaieda, S. Hama, H. Yamaji, A. Kondo, E. Izumoto, H. Fukuda, Biochem. Eng. J. 23 (2005) 5–51.
[7] W. Du, Y.Y. Xu, D.H. Liu, Z.H. Li, J. Mol. Catal. B: Enz. 37 (2005) 68–71.
[8] W. Li, W. Du, D.H. Liu, Process. Biochem. 42 (2007) 1481–1485.
[9] Y. Wang, H. Wu, M.H. Zong, Bioresour. Technol. 99 (2008) 7232–7237.
[10] Z. Guo, Y. Sun, Food Chem. 100 (2007) 1076–1084.
[11] S.F. Dias, A.C. Correia, M.M.R. Fonseca, J. Mol. Catal. B: Enz. 21 (2003) 71–80.
[12] S. Murgia, F. Caboi, M. Monduzzi, H.L. Wahren, T. Nylander, Progr. Colloid Polym. Sci. 120 (2002) 41–46.
[13] J.A. Laszlo, D.L. Compton, K.E. Vermillion, J. Am. Oil Chem. Soc. 85 (2008) 307–312.
[14] A.M. Fureby, L. Tian, P. Adlercreutz, Enzyme Microb. Technol. 20 (1997) 196–206.
[15] A.F. Vikbjerg, H. Mu, X. Xu, J. Mol. Catal. B: Enz. 36 (2005) 14–21.
[16] M. Oda, M. Kaieda, S. Hama, H. Yamaji, A. Kondo, E. Izumoto, H. Fukuda, Biochem. Eng. J. 23 (2005) 45–51.
[17] A. Ducret, M. Trani, R. Lortie, Enzyme Microb. Technol. 22 (1998) 212–216.
[18] T.K. Yang, Food Chem. 92 (2005) 101–107.