The catalytic properties and mechanism of cyclohexane/DBSA/water microemulsion system for esterification

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Abstract

A model esterification reaction of hexanol and hexanoic acid in the cyclohexane/dodecylbenzenesulfonic acid (DBSA)/water microemulsion system has been investigated. Compared with cyclohexane/AOT/water microemulsion system and organic medium system, esterification reaction in the cyclohexane/DBSA/water system can perform relatively rapidly whether catalyzed by *Candida cylindracea* lipase (Ccl) or not, this demonstrated that DBSA itself can act as a kind of acid catalyst. Comparison of conversion in several acid-catalyzed reaction systems were also performed and the results showed that the conversion in DBSA system was the most highest, which proved the key factor affecting the conversion was not the acid strength, but the attribute of DBSA as a kind of surfactant. Furthermore, we also perform transesterification reaction of butanol and ethyl butyrate (and methyl butyrate) in the DBSA reverse microemulsion system; however, it cannot get remarkably high conversion like esterification, this reason may be alcohol produced during the transesterification cannot easily enter water droplet like water produced by the esterification. Comparison of conversion in the DBSA system with conversion in the O/W emulsion system DBSA as an emulsion agent also indicated that the conversion in the DBSA reverse microemulsion was much higher. Finally, the mechanism of reaction was also explored. After realizing that DBSA is both acid catalyst and surfactant, we concluded that it was concurrent function of acid catalyst and surfactant that played an important role in improving reaction rate and conversion of esterification.
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1. Introduction

It is well known that the direct esterification of carboxylic acid and alcohol, and transesterification of ester and alcohol play important roles in the production of organic esters [1,2]. But to enhance the reaction rate and conversion, we must either make one of the reactants excess or remove the formed alcohol (or water) from products so as to promote the equilibrium process to shift towards the products side. At present, most of the works reported in the literatures have been based on esterification reactions using long-chain length fatty acids and alcohols or short-chain fatty acids and alcohols [3–7]. Catalysts that can be used to catalyze the esterification and transesterification have two main kinds of catalyst, one is chemical catalyst and the other is biocatalyst.

Enzymes are a kind of biocatalyst and are efficient catalyst in synthetic chemistry, their catalytic activity with unnatural substrates attracting much attention because of their much milder reaction conditions and friendly environments [8–14]. In recent years, one of the most intensively studied areas has been the technique of entrapping enzymes in reverse micelles or microemulsion [15,16].

A microemulsion is a thermodynamically stable, isotropic, optically transparent solution consisting of water, oil and a surfactant. Often, the formation of microemulsion requires the presence of a co-surfactant. Depending on the microstructure, microemulsion can be divided into three main cases: an oil-in-water (O/W), a bicontinuous structures, an water-in-oil (W/O) that is aqueous droplets (stabilized by surfactant)

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is dispersed in a continuous organic medium (also called as reverse micelles). For enzyme-catalyzed reactions, a W/O type is usually used. The enzyme is solubilized in the water droplets of the microemulsion, while the oil-soluble substrates are dissolved in the continuous, organic phase. The reactions take place at the oil/water interface and the products are distributed to the oil phase or the water phase [17]. This kind of system mainly has two advantages: (1) lipase molecules can be entrapped in water-containing microdrops, avoiding direct contract with unfavorable organic medium and retaining their catalytic activity. (2) Larger polar/apolar interfacial area improves the interaction between the enzyme and substrates [13–15].

In our work, because reactant hexanol and hexanoic acid are polar organic solvent, they can orient at interface to act as co-surfactant. Although both hexanol and hexanoic acid will decrease with reaction proceeding, there should be a little remanent reactant in the reaction system. So, we think it is more appropriate to call the reaction system as reverse microemulsion system (in short: microemulsion system) in this paper. In the following, we reserve the term microemulsion to our reaction system.

Surfactant is a very important factor in formation of microemulsion system, most of the enzymatic reactions were performed in microemulsion or reverse micelles stabilized by AOT and CTAB surfactant [18–23], few attention has been paid to the other surfactants. So, we initiated our experiment to use DBSA, a relatively cheap and widely used commercial surfactant to form reverse microemulsion. Based on above mentioned, a model esterification reaction of hexanol and hexanoic acid, which was catalyzed by *Candida cylindracea* lipase (Ccl) in DBSA/cyclohexane microemulsion system, was undertaken to compared lipase activity in this system with AOT/cyclohexane microemulsion system and cyclohexane system.

To us surprised, the results of our experiments showed that even if at mild conditions, esterification reaction in the DBSA system can perform very rapidly and obtain relatively considerable conversion whether catalyzed by *C. cylindracea* or not as compared with AOT microemulsion system and organic medium system, this result encouraged us to further explore the reaction mechanism. According to our investigation, we concluded that relatively high conversion in DBSA system is ascribed to the two-fold functions of DBSA, which are surfactant as well as acid catalyst. So, this proved that DBSA microemulsion should be a novel and promising esterification system in improving reaction rate and conversion under relatively mild reaction conditions.

2. Experimental

2.1. Chemicals

Lipase from *C. cylindracea* (specific activity 2.0 unites/mg) was purchased from Fluka; dodecylbenzenesulfonic acid (DBSA, ≈97% purity) was purchased from Tokyo Kasei Kogyo Co. Ltd. (Japan), sodium 1,4-bis (2-ethylhexyl) sulphosuccinate (AOT, ≈96% purity) was purchased from ACROS organics. 2-Naphthalenesulfonic acid (2-NTSA), sodium dodecylbenesulfonic acid (SDBS) and *p*-toluenesulfonic acid (TsOH) were, respectively, obtained from Sigma–Aldrich Co. Ltd. (Germany), chemical factory of Beijing and Xizhong chemical factory of Beijing (China). Hexanoic acid, hexanol, butanol and *n*-ethyl butyrate were all from Shanghai chemical reagent factory (China). Sulfuric acid (H_2SO_4), cyclohexane, *n*-methyl butyrate and *n*-butyl acetate were purchased from Tianjin chemical reagent Co. Ltd. (China). All the other chemicals used were of analytical grade and were used without further purifications.

2.2. Preparation of microemulsion

Microemulsion were prepared by the addition the desired concentration of DBSA (or AOT) to cyclohexane (10 ml). In this mixture, a relevant concentrations buffer (pH 7.0) were then added to, and the final w_0 value was adjusted by the addition of the amount of the buffer. The mixture was briefly shaken until an optically clear single-phase solution was formed.

2.3. Reaction in microemulsion

Both synthesis of esters and transesterification were carried out using above microemulsion by addition of 0.5 mmol hexanol (ethyl butyrate or methyl butyrate) and 1 mmol hexanoic acid (or butanol), the appropriate amounts of lipase was added to initiate reaction. The reaction systems were stirred continuously and incubated at 40 °C for certain times. After some time, samples (200 μl) were withdrawn from the reaction medium, dissolved with ethanol to stop any enzymatic reaction, and then analyzed subsequently by GC.

2.4. Reaction in cyclohexane

The procedure is the same to reaction in microemulsion except that there is not any surfactant (DBSA or AOT) added.

2.5. Reaction in O/W emulsion

In a typical experiment of esterification in O/W emulsion. O/W emulsion was prepared by the addition of amount of DBSA to water (10 ml), then 0.5 mmol hexanol (ethyl butyrate or methyl butyrate) and 1 mmol hexanoic acid (or butanol) were added to the above mixture. The mixture was briefly shaken until white turbid was formed. The procedure performed afterward was the same to the microemulsion.

2.6. Chromatographic analysis

Samples were monitored on a Varian CP-3380 Gas Chromatograph equipped with a FID detector using an Agilent

Technologies HP-5 column (15 m × 0.530 mm × 1.50 μm). The temperature program used begins with 40 °C and isothermal hold at 40 °C for 2 min, then increased to 100 °C at a gradient of 5 °C/min, followed by an isothermal hold at 100 °C for 2 min, subsequently 100–250 °C at a rate of 30 °C/min, at which temperature the column was maintained for 20 min; injection port temperature is 200 °C; detector temperature is 250 °C; carrier gas is nitrogen. Internal standard solution (5 μl) was mixed into above sample, then 0.5 μl samples were injected in the GC. The conversion percent of hexanol to its *n*-hexylhexaneate was defined as the consumption of hexanol divided the initial amount of hexanol (the definition of the conversion percent of ethyl butyrate and methyl butyrate to their butyl butyrate obeyed the same rule).

3. Results and discussion

3.1. The time course

The time courses for the direct esterification of hexanol with hexanoic acid by Ccl in the three different systems were shown in Fig. 1. The conversion of hexanol increased linearly with increasing reaction time in DBSA system. A similar pattern was observed in AOT system. But in organic medium, conversion nearly kept unchanged after 48 h, this is probably because Ccl in organic solvent is deactivated, whereas in DBSA system and AOT system, activity of Ccl can retain for a relatively longer time. The similar results were also obtained in the other literatures [19–21].

As shown in Fig. 1, DBSA system exhibited the maximal reaction rate of hexanol. Esterification took place rapidly in the first 6 h and quickly reached reaction equilibrium with esterification degree of 92.35% after 12 h, which was markedly higher than in the other two systems. For exam-
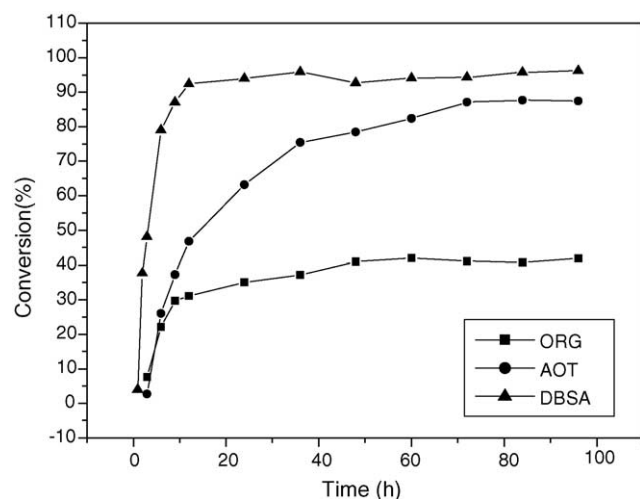
ple, the conversion of 46.83 and 32.11% were obtained after 12 h in AOT system and organic solvent, respectively. It has been recognized that DBSA is also one of Brönsted acid catalyst [24–27]; therefore, there existed two kinds of catalyst in reaction system. Because of collaboration of these two kinds catalyst, reaction rate of esterification is remarkably higher than the other two systems.

3.2. Contrast of conversion in DBSA microemulsion with lipase present and absent

Which catalyst is much more effective on accelerating reaction rate during reaction course? This problem urged us to perform experiment to make a comparison of conversion in DBSA microemulsion with lipase present and absent. The time courses of esterification reaction in two systems were shown in Fig. 2.

From Fig. 2, we can observe that the difference between two systems existed only before 6 h and conversion was nearly the same after that, but the conversion in DBSA microemulsion with lipase was slightly higher than DBSA system without lipase. This result showed that lipase could increase reaction rate in a relatively short time; however, DBSA could increase both reaction rate and the conversion during the whole reaction course. So, we can confirm that DBSA played important role in esterification as acid catalyst. In the absence of lipase, the reality that high conversion still can be obtained in DBSA microemulsion system is favorable to economy due to relatively high price of lipase.

3.3. Comparison of conversion in several acid-catalyzed reaction systems

Subsequently, we studied the esterification in the other several acid-catalyzed systems with the same acid concen-

Fig. 1. Effect of reaction time on conversion of exanol. Reaction conditions: hexanol:hexanoic acid = 1:2 (molar ratio), enzyme amount = 30 mg, T = 313 K, pH 7.0, w_0 = 6 (for reverse microemulsion), water content = 5 μl (for organic solvent).
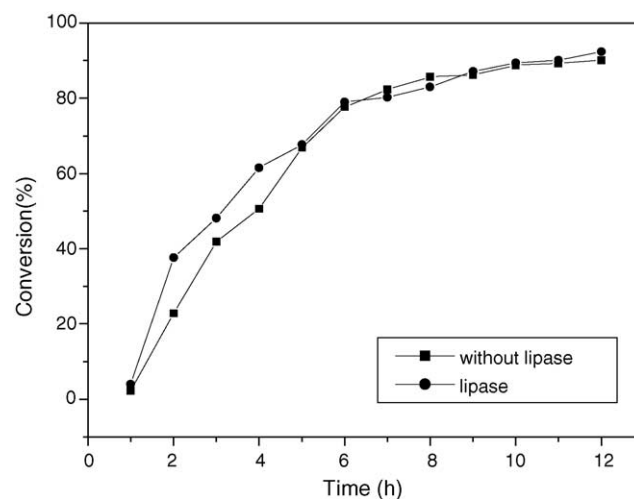

Fig. 2. Contrast of cyclohexane/DBSA/water microemulsion with lipase present and absent. Reaction conditions: hexanol:hexanoic acid = 1:2 (molar ratio), enzyme amount = 30 mg, pH 7.0, t = 6 h, w_0 = 6, T = 313 K.

Table 1
Esterification in cyclohexane with various catalysts

Entry	Catalyst	Conversion (%)
1	DBSA	77.64
2	SDBS (pH 3.2)	8.00
3	TsOH	12.79
4	2-NTSA	21.00
5	H_2SO_4	0.00

Reaction conditions: hexanol:hexanoic acid = 1:2 (molar ratio), pH 7.0, $t = 6$ h, $w_0 = 6$, $T = 313$ K, catalyst concentration: 50 mmol l^{-1}.

tration to explore whether DBSA surfactant possess special characteristic as acid catalyst.

From Table 1, it is evident that after proceeding 6 h of reaction, the conversion in DBSA microemulsion system is much higher than the other acid-catalyzed systems with the same acid catalyst concentration of 50 mmol l^{-1}. It proved that DBSA should be the most efficient catalyst. Because most of the acid catalysts required high temperature environment to exert their high catalytic activity [28–31], comparatively, DBSA can exert high conversion even if in room temperature environment. The reality that reaction rate was still very rapid at relatively mild conditions revealed the superiority of DBSA. We thought that this superiority should be attributed to the property of DBSA as a kind of surfactant. It is not difficult to infer that the long-alkyl chain is crucial for the efficient catalysis because it enhances the hydrophobic of DBSA surfactant, which make it possible that DBSA molecules can form the microemulsion more easily. This will lead to polar heads of DBSA surfactant that has certain acidity to congregate; hence, a relatively high acidity was attained in aqueous microdomain to accelerate esterification more efficiently.

3.4. The time course of transesterification

We also studied whether DBSA microemulsion system was applicable to the transesterification reaction. Time course of transesterification reaction of butanol and ethyl butyrate (methyl butyrate) in DBSA system were shown in Fig. 3.

Fig. 3 shows that the conversion of ethyl butyrate and methyl butyrate increased linearly with increasing reaction time, but obviously the conversion of ethyl butyrate nearly kept constant after 12 h. Both conversion of methyl butyrate and ethyl butyrate were lower than esterification, this results demonstrated that transesterification in the DBSA system cannot get remarkably high conversion like esterification, this reason may be there is water produced in the esterification and produced water can enter aqueous microdomain of microemulsion, which promote the equilibrium process in favor of the products side; whereas alcohol produced during transesterification cannot easily enter water droplet like water due to the distribution limitation between organic media and water microdomain; moreover, the volume of organic solvent is far more larger than volume of water. The difference existed
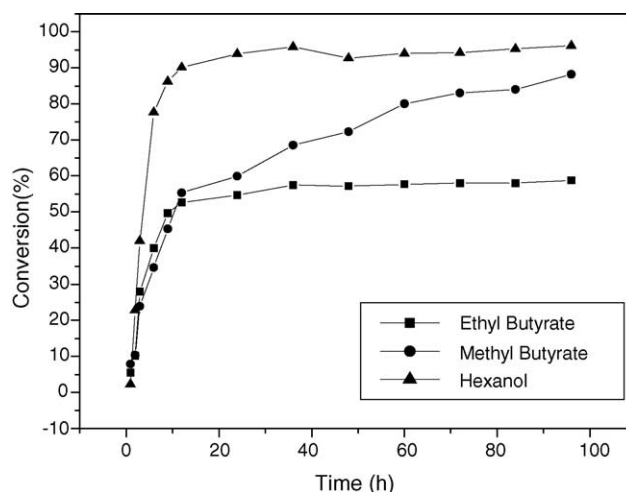

Fig. 3. Effect of reaction time on conversion of ethyl butyrate and methyl butyrate. Reaction conditions: ethyl butyrate (methyl butyrate):butanol = 1:2 (molar ratio), $T = 313$ K, pH 7.0, $w_0 = 6$.

between methyl and butyrate ethyl butyrate is because the former produced methanol enters water droplets of microemulsion relatively easier than the latter produced ethanol due to distribution ratio of methanol between water and organic solvent is much larger than the ethanol. This verified that the hydrophilic of product is a key factor of increasing reaction conversion, the higher the hydrophilic of product, the easier of product enters water microdomain, which will accelerate reaction to form esters.

3.5. Contrast between O/W emulsion and DBSA system

Esterification in O/W emulsion was also performed as compared with DBSA microemulsion and the results were shown in Table 2. The results demonstrated that the difference of conversion between O/W emulsion and DBSA reverse microemulsion existed. The conversion in DBSA microemulsion was higher than O/W emulsion both 6 and 24 h. It is true that emulsion forms the hydrophobic interior where substrate disperse [32]; however, substrate cannot enter interior entirely because of the limitation of dispersion, which will lead to the lower conversion. On the contrary, substrate is readily soluble in the bulk oil phase of DBSA microemulsion, so small amount water produced in the reaction course can easily enter water interior.

Table 2
Esterification in O/W emulsion and DBSA system

Entry	Reaction system	Time (h)	Conversion (%)
1	O/W emulsion	6	36.19
2		24	53.29
3	DBSA system	6	77.64
4		24	93.95

Reaction conditions: hexanol:hexanoic acid = 1:2 (molar ratio), pH 7.0, $t = 6$ h, $w_0 = 6$ (for reverse microemulsion), $T = 313$ K.
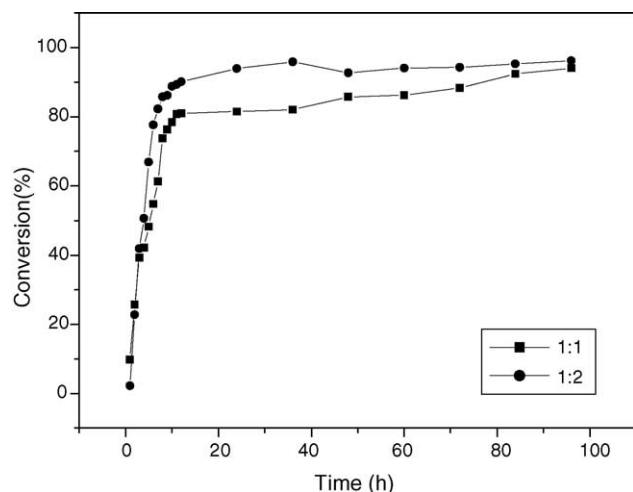
Fig. 4. Effect of molar ratio of substrate on conversion of hexanol. Reaction conditions: pH 7.0, $t = 6$ h, $w_0 = 6$, $T = 313$ K.

3.6. Effect of molar ratio of substrate on conversion of esterification

Effect of molar ratio of substrate on conversion of esterification was studied to testify the superiority of DBSA microemulsion system. The result was shown in Fig. 4. It proved that the conversion was still very high even if the molar ratio of substrate is 1:1. Very high conversion can be obtained in DBSA microemulsion even if either of reactants is not excessive, which verified that DBSA microemulsion system is a very powerful reaction medium for esterification.

3.7. Mechanism of reaction

The results of the above experiments encouraged us further explored the reaction mechanism. In the DBSA microemulsion systems, esterification took place at interface area and water produced by esterification would enter water microdomain, which made esterification equilibrium shift towards the product. According to the results of calculation, we found when conversion was 100%, the water produced during esterification would be only 9 μl (the same to the one w_0 value). Produced water is so little that it can easily enter the water microdomain in microemulsion, which will enlarge the water droplet in microemulsion. The concept of esterification in DBSA-based microemulsion was shown in Fig. 5.

Based on above results of investigation, we concluded that the reaction mechanism of DBSA system was as follows: (1) DBSA itself is a kind of surfactant, so it can form thermodynamics stable microemulsion system, which can provides a high interfacial area of contact to increase reaction rate. (2) DBSA itself is also a kind of acid catalyst. Because of formation of microemulsion, polar heads of DBSA surfactant that has certain acidity will congregate, which lead to relatively high acidity in aqueous microdomain, so this can catalyze esterification more effectively. (3) The water microdomain in
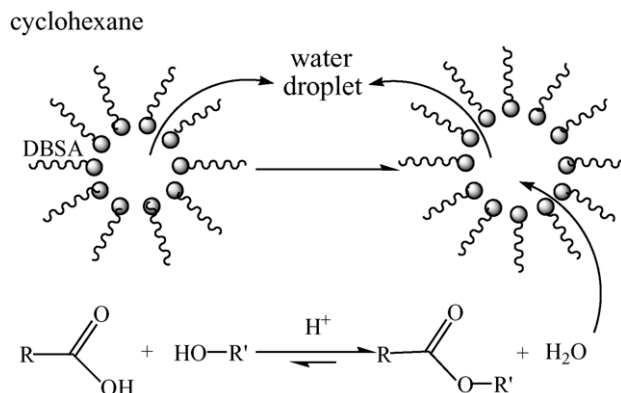

Fig. 5. Illustration of direct esterification in cyclohexane/DBSA/water microemulsion.

DBSA microemulsion can entrap water produced by esterification, which would accelerate the formation of ester and promote the equilibrium process in favor of the products side.

4. Conclusions

We have described esterification in cyclehexane/DBSA/water microemulsion and compared it to the cyclehexane/AOT/water microemulsion and organic solvent. An important point to mention is that in DBSA microemulsion system whether catalyzed by Ccl or not, very high conversion (>90%) of *n*-hexanol to its *n*-hexylhexaneate was obtained only after 12 h. This high conversion obtained in a relatively short time justified the reaction performed very quickly, which proved that DBSA microemulsion should be a very promising reaction system because of its two-fold functions; firstly, it can act as a Brönsted acid to catalyze the reaction and secondly, it can form microemulsion as surfactant to enlarge interface area and entrap water produced during esterification reaction. In addition, this system has three main advantages: (1) no requirement for removal of the formed water. (2) High conversion can be obtained under mild conditions. (3) This system was found still efficient even if substrate ratio is 1:1, so this did not require either of reactants is excessive. In this paper, we merely consider short-chain fatty acids and alcohols, but we think that DBSA microemulsion also should be applicable to the esterification of long-chain length fatty acids and alcohols; presently, we dedicate our work to them and detailed studies of effect of reaction parameters such as w_0 ([H$_2$O]/[surfactant]), pH of buffer solution and system temperature on conversion is proceeding.

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