J. Microbiol. Biotechnol. (2016), 26(1), 80–88
http://dx.doi.org/10.4014/jmb.1506.06075

jmb

Enzyme-Catalyzed Henry Reaction in Choline Chloride-Based Deep Eutectic Solvents

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Received: July 1, 2015
Revised: September 11, 2015
Accepted: October 5, 2015

First published online
October 6, 2015

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Supplementary data for this paper are available on-line only at http://jmb.or.kr.

pISSN 1017-7825, eISSN 1738-8872

The enzyme-catalyzed Henry reaction was realized using deep eutectic solvents (DESs) as a reaction medium. The lipase from *Aspergillus niger* (lipase AS) showed excellent catalytic activity toward the substrates aromatic aldehydes and nitromethane in choline chloride:glycerol at a molar ratio of 1:2. Addition of 30 vol% water to DES further improved the lipase activity and inhibited DES-catalyzed transformation. A final yield of 92.2% for the lipase AS-catalyzed Henry reaction was achieved under optimized reaction conditions in only 4 h. In addition, the lipase AS activity was improved by approximately 3-fold in a DES–water mixture compared with that in pure water, which produced a final yield of only 33.4%. Structural studies with fluorescence spectroscopy showed that the established strong hydrogen bonds between DES and water may be the main driving force that affects the spatial conformation of the enzyme, leading to a change in lipase activity. The methodology was also extended to the aza-Henry reaction, which easily occurred in contrast to that in pure water. The enantioselectivity of both Henry and aza-Henry reactions was not found. However, the results are still remarkable, as we report the first use of DES as a reaction medium in a lipase-catalyzed Henry reaction.

Keywords: Lipase AS, Henry reaction, deep eutectic solvents

Introduction

The Henry reaction is an important and classical C–C bond-formation reaction, which is an essential element of synthetic organic chemistry [7, 16, 18, 19]. The reaction is the coupling of a nucleophilic nitroalkane with an electrophilic aldehyde or ketone to produce a synthetically useful β-nitro alcohol [18]. The resulting β-nitro alcohols have been employed in the synthesis of many key intermediates of biologically active compounds, such as natural products, insecticides, fungicides, and antibiotics [2, 3, 6, 16]. The Henry reaction can occur using most popular organic bases, including carbonates, bicarbonates, alkali metal hydroxides, alkoxides, and organic nitrogen bases [18]. Apart from bases, some other catalysts include the rare earth metal alkoxides, rare earth hexamethyldisilazides, and binaphthol-rare earth metal complexes [18]. The main drawbacks of the synthetic reactions are the formation of unwanted byproducts that pollute the environment [4]. Therefore, the determination of mild reaction conditions that prevent the formation of unwanted byproducts is necessary.

Biocatalysts show immense advantages, including mild reaction conditions, simple separation, good selectivity, and high yields, as efficient and environmentally friendly biotransformation tools in organic synthesis [27]. A growing number of biocatalysts have been found to be capable of catalyzing synthetic reactions, deviating from their natural functions. Exploiting biocatalyst promiscuity is believed to have great potential in expanding synthetic methodologies [13]. Some biocatalysts show good catalytic activity for the Henry reaction. Although some enzymes, such as hydroxynitrile lyase, are able to induce some degree of enantioselectivity [14, 17], the use of aminoacylase, transglutaminase or lipase generally leads to poor enantiocontrol [5, 24–26]. In this case, enantioselectivity is

only obtained by coupling the biocatalytic Henry reaction to the enzymatic kinetic resolution of the racemic mixture [18]. However, even when low enantioselectivity may be achieved, biocatalytic processes present clear advantages from an environmental point of view. Some biocatalysts have been described to promote the Henry reaction in organic solvents, biphasic systems composed of water and organic solvents, or in aqueous medium [25, 26]. However, the environmental harm from organic solvents is unavoidable. In addition, the long reaction time, high reaction temperature, and low enzymatic activity are unfavorable. Therefore, developing new enzymatic methods for the Henry reaction in eco-friendly and effective reaction media is of great importance.

An environmentally friendly, enzyme-promoted procedure for the Henry reaction was first studied using water-in-[Bmim][PF_6] microemulsions as the reaction medium. The Amano acylase from *Aspergillus oryzae* showed good catalytic activity for the addition reactions of nitromethane with a series of aromatic aldehydes; however, reaching the ideal reaction yield was still time-consuming (48 h) [29]. Although ionic liquids are regarded as potential environmentally friendly alternatives to conventional organic solvents owing to their unique properties, such as excellent solubility as well as thermal and chemical stabilities, their high price and operational difficulties limit their usage [20,22].

In recent years, new types of solvents, namely, deep eutectic solvents (DESs), have been used as reaction media [1, 23]. DESs, similar to ionic liquids, have a low melting point, low volatility, and high thermal stability. In addition, DESs are biodegradable, non-toxic, inexpensive, and simple to prepare. DESs consist of an ammonium or phosphonium salt, such as choline chloride, and a hydrogen-bond donor (HBD), such as urea or glycerol. The properties of DESs can be easily changed by adjusting the type and molar ratio of the salt and HBD [10]. Although the strong hydrogen bonding involved in this kind of solvent might be extremely denaturing for enzymes, some studies have demonstrated that some hydrolases can maintain their high activities in DESs, and the DESs can even improve the catalytic activity of the enzymes [8, 10, 12]. However, only a very few of reaction types such as esterification or transesterification dealing with their use in DESs have been reported so far [10, 12]. Thus, the applications of DESs in other enzyme-catalyzed reactions have attracted increasing research attention.

In this study, we introduced DESs in an enzyme-catalyzed Henry reaction to promote the reaction. We report the first examples of biocatalytic Henry reactions in DES-based medium and provide a rapid, eco-friendly synthesis strategy for nitro alcohol compounds.

Materials and Methods

Materials

Lipase from *Aspergillus niger* (Lipase AS "Amano") was donated by Amano Enzyme Ltd., Japan. Choline chloride (ChCl) was bought from J & K Scientific Ltd., China. Benzaldehyde, 2-nitrobenzaldehyde, 3-nitrobenzaldehyde, and 4-nitrobenzaldehyde were purchased from Shanghai Darui Fine Chemical Ltd., China. 2,4-Dinitrobenzaldehyde and ethylene glycol (EG) were purchased from Energy Chemical, China. 4-Bromobenzaldehyde and 4-methylbenzaldehyde were bought from Aladdin Industrial Co., China. Nitromethane was purchased from Sinopharm Chemical Reagent Ltd., China. Glycerol (Gly) and Urea were purchased from Changchun Baijin Biotechnology Ltd, China. All organic solvents were reagent grade and employed without further purification.

Preparation of Deep Eutectic Solvents

In this study, DESs were prepared according to the procedures reported in the literature [1, 10]. The preparation involved reaction of choline chloride with urea, glycerol, or ethylene glycol at 80°C until a clear solution was obtained. The prepared DESs were cooled and used for the enzyme-catalyzed Henry reaction without any purification.

General Procedure for Lipase AS-Catalyzed Henry Reaction in DES

4-Nitrobenzaldehyde (0.15 mmol) and nitromethane (1.5 mmol) were added to DES (0.7 ml) in a sealed flask. The lipase AS (10 mg) was then added to the reaction mixture. The resulting mixture was shaken at 25°C under nitrogen protection. When the aldehyde substrate was entirely converted, water (0.7 ml) was added into the reaction mixture to reduce the viscosity of the reaction system, and dichloromethane was used for the extraction. The resulting extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in minimal ethyl acetate and loaded onto a silica gel column. The products were monitored on silica gel plates (Qingdao Haiyang Chemical Co., Ltd., China) using UV light for spot detection. All the purified products were eluted with ethyl acetate:petroleum ether (1:6 (v/v)). The samples were collected, concentrated, and weighed to calculate the reaction yield. The authenticity of the organic compounds prepared during the study was confirmed by 300 MHz nuclear magnetic resonance (Mercury-300B; Varian, USA). The enantiomeric excesses of the Henry products were analyzed by high-performance liquid chromatography (HPLC,

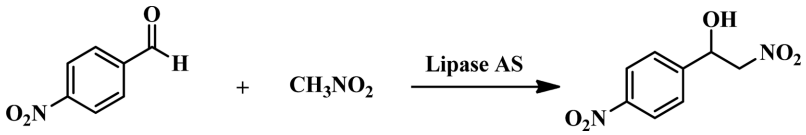


Fig. 1. Lipase AS-catalyzed Henry reaction of 4-nitrobenzaldehyde and nitromethane.

Acme 9000; Young Lin Instrument Co., Ltd.) using a Daicel Chiralpack AD-H or Daicel Chiralpack OD-H column. The elution solvent was a mixture of *n*-hexane and isopropanol. The flow rate was 1.0 ml/min, and the detection was achieved under UV light at 210 nm. The retention times for all the products were confirmed based on their standard (purity >99.5%). Control reactions without enzyme were carried out under the same conditions. The NMR and HPLC spectra for all the products are shown in the Supplementary material.

General Procedure for Lipase AS-Catalyzed Aza-Henry Reaction in DES with Water

The aza-Henry reaction of *N*-Ts (Ts = *p*-tosyl) imines (0.15 mmol) coupling with nitromethane (2.25 mmol) was catalyzed by lipase AS (10 mg) in 0.7 ml of ChCl:Gly (1:2) with different vol% water. The resulting mixture was shaken at 25°C under nitrogen protection. When the *N*-Ts imines substrate was entirely converted, water (0.7 ml) was added into the reaction mixture to reduce the viscosity of the reaction system, and ethyl acetate was used to extract the products. The resulting extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. After the samples were dissolved in 2.0 ml of acetonitrile, a 20 µl aliquot of the sample was obtained and analyzed by HPLC (Acme 9000, Young Lin Instrument Co., Ltd.) using an Eclipse XDB-C18 column. The elution solvent was a 50:50 mixture of acetonitrile and water. The flow rate was 1.0 ml/min, and the detection was achieved under UV light at 254 nm. The retention time of the corresponding nitro amine product was 7.7 min. The yields of nitro amine products were confirmed by external standard method based on their standard (purity >99.5%). Control reactions without enzyme were carried out under the same conditions. The NMR and HPLC spectra for the product are shown in the Supplementary material.

Fluorescence Spectroscopy

Fluorescence measurements were carried out using a RF-5301 PC spectrofluorophotometer. Enzyme solution was prepared by dissolving lipase AS (20 mg) in deionized water (1.0 ml), and then a 100 µl enzyme solution was mixed with DES (ChCl: Gly 1:2) and water to reach a final water content that ranged from 20 to 100 vol%. The enzyme samples prepared were detected by their fluorescence spectrum. The fluorescence was recorded at an excitation wavelength of 280 nm, and the detection wavelength ranged from 300 to 540 nm. A blank medium without enzyme was subtracted to discount the influence of the DES and water on the enzyme fluorescence spectrum.

Results and Discussion

Effects of Various DESs on Lipase AS-Catalyzed Henry Reaction

A model reaction of 4-nitrobenzaldehyde with nitromethane catalyzed by lipase AS was selected as a standard (Fig. 1) to determine whether DESs could be a suitable medium for enzyme-catalyzed Henry reactions. We selected three DESs, including ChCl:Gly, ChCl: EG, and ChCl:Urea, to observe their effects on a lipase AS-catalyzed Henry reaction. The molar ratio of ChCl and HBD was set at 1:2 to ensure that the formed DES was liquid at our reaction temperature (25°C). The possible catalytic effect of DES medium was studied as control experiments to ensure the accuracy of the experimental results. The results show that the Henry reaction could proceed in DES without any catalyst. Thus, DES might play an important role in the Henry reaction through hydrogen bonding interactions with nitromethane and aldehyde group. The final yields of enzyme-catalyzed Henry reactions were calculated based on the control experiments. As shown in Table 1, the lipase AS-catalyzed Henry reaction occurred in all of the selected DESs to

Table 1. Effects of various DESs on lipase AS-catalyzed Henry reaction[a].

DESs	Molar ratio of ChCl to HBD	Time (h)	Yield (%)[b]	Yield (%)[c]	Yield (%)[d]
ChCl:Gly	1:2	4	19.9	52.2	32.3
	1:5	4	8.3	34.5	26.2
	1:8	4	6.7	31.3	24.6
	1:10	4	4.7	27.9	23.2
ChCl:EG	1:2	4	38.9	54.4	15.5
	1:5	4	29.8	44.3	14.5
	1:8	4	27.5	41.2	13.7
	1:10	4	23.8	38.9	15.1
ChCl:Urea	1:2	0.25	25.3	28.2	2.9

[a]*Reaction conditions*: Lipase AS (10 mg), 4-nitrobenzaldehyde (0.15 mmol), nitromethane (1.5 mmol), and DES (0.7 ml) were stirred at 25°C.

[b]Total yields of Henry reaction without enzyme.

[c]Total yields of Henry reaction with enzyme.

[d]Final yields of enzyme-catalyzed Henry reaction.

different extents depending on the nature and molar ratio of DES components (*i.e.*, ChCl and HBD). This finding was attributed to possible variations in the network formed by hydrogen bonds, leading to different behaviors of their constituents. When ChCl:Gly was used as the reaction medium, the total reaction yields of both the lipase AS-catalyzed reaction and the control experiment decreased with the increase in glycerol content; this finding might be attributed to the resulting high viscosity of DES, thereby hindering the substrate from accessing the enzyme active site and decreasing the reaction rate [25]. The best final reaction yield of 32.3% was obtained with ChCl:Gly (1:2) as the reaction medium. Similar results were also observed when ChCl:EG was employed as the reaction medium, but the DES-catalyzed transformation was much stronger than that in ChCl:Gly, leading to a low final reaction yield of lipase AS-catalyzed Henry reaction (13.7% to 15.5%). For ChCl:Urea, we found that the yield of lipase AS-catalyzed Henry reaction was similar to that of DES-catalyzed transformation. This phenomenon could be explained by a previous research of Singh *et al.* [23], in which the ChCl:Urea itself was found to be a highly effective catalyst of the Henry reaction, restricting lipase activity. From the above results, we concluded that the lipase AS-catalyzed Henry reaction can be achieved in DES, but DES-catalyzed transformation was the major obstacle for its application. Thus, other strategies are needed to control the DES-catalyzed transformation.

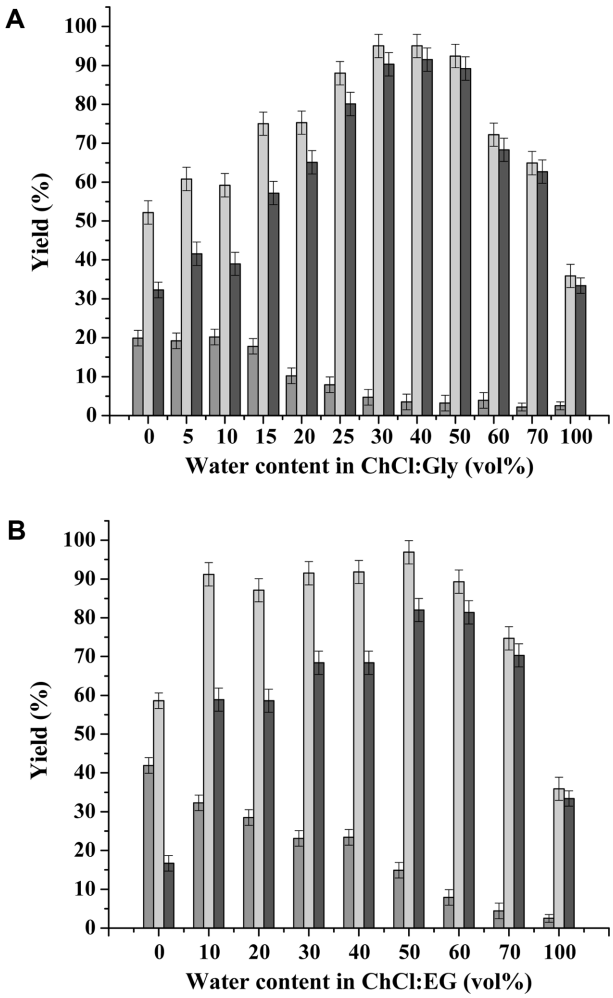


Fig. 2. Effects of water content on lipase AS-catalyzed Henry reactions of 4-nitrobenzaldehyde and nitromethane in (**A**) ChCl:Gly (1:2) and (**B**) ChCl:EG (1:2).
■ Total yields of Henry reaction without enzyme. ■ Total yields of Henry reaction with enzyme. ■ Final yields of enzyme-catalyzed Henry reaction.

Effects of Water Content on Lipase AS-Catalyzed Henry Reaction in DES

Enzymes require a specific amount of water to maintain their activities. In this experiment, water was introduced in DES to determine its effect on the lipase AS-catalyzed Henry reaction. From Fig. 2, DES-catalyzed transformation was controlled effectively, and the rates of lipase AS-catalyzed reactions were drastically accelerated with the increase in added water. The maximum final reaction yields of 90.3% and 91.5% were achieved when 30 and 40 vol% water, respectively, were added to ChCl:Gly (1:2), which only needed 4 h to reach such high reaction yields. However, adding more than 40 vol% water decreased the lipase AS-catalyzed conversions, although the DES-catalyzed transformation was weak. When pure water was selected as the reaction medium for the lipase AS-catalyzed Henry reaction, the final reaction yield was only 33.4% in the same reaction conditions as that in the DES–water mixture. Similar results were observed when ChCl:EG was used as the reaction medium, and a high final yield of 82% was reached with 50 vol% water in ChCl:EG (1:2).

The above results indicate that ChCl-based DES with water can be an effective reaction medium to enhance lipase AS-catalyzed Henry reaction. This phenomenon might be explained by the formation of strong interactions (hydrogen bonds) between water and DES, thus making DES a suitable solvent rather than a catalyst. Consequently, the hydrogen bonds formed in the DES–water mixtures constitute the main driving force affecting the spatial conformation of the enzyme, further leading to a change in lipase activity [9, 28]. Fluorescence spectroscopy results of lipase AS in ChCl:Gly (1:2) with water are shown in Fig. 3.

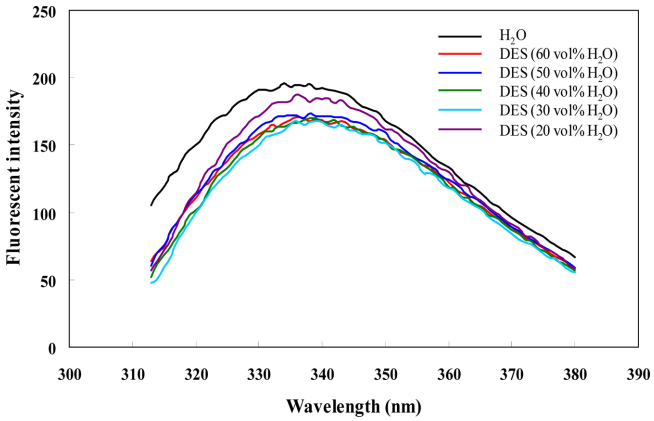


Fig. 3. Fluorescence spectroscopy of lipase AS in ChCl:Gly (1:2) with water.

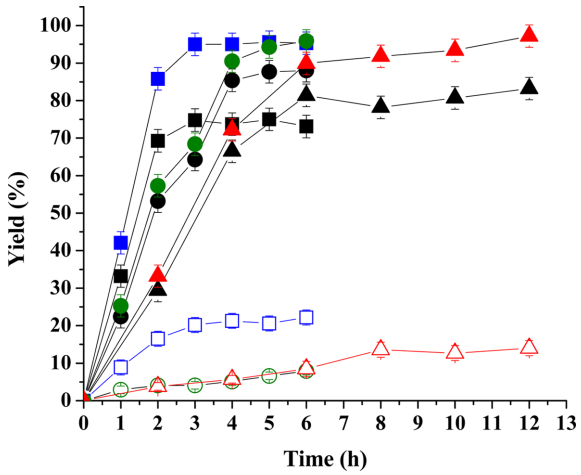


Fig. 4. Effects of temperature on the time-course yields of lipase AS-catalyzed Henry reactions in ChCl:Gly (1:2) with 30 vol% water.

Total yields of Henry reactions without enzyme at 20°C (△), 25°C (○), and 30°C (□). Total yields of Henry reactions with enzyme at 20°C (▲), 25°C (●), and 30°C (■). Final yields of enzyme-catalyzed Henry reactions at 20°C (▲), 25°C (●), and 30°C (■).

The decrease of water content in DES can trigger a λ_{max} redshift; a significant λ_{max} redshift from 334 nm to 339 nm was observed when 30 vol% or 40 vol% water was added to DES, which agreed well with the experimental data. This result suggested that an appropriate amount of water in DES can trigger a subtle change in the microenvironment of the amino acid residues in lipase AS and is capable of providing the enzyme with a slightly more relaxed tertiary structure, possibly facilitating the lipase-catalyzed Henry reaction. Excessive water (>40 vol%) might constitute a diffusion barrier for the substrates, thus decreasing both lipase AS- and DES-catalyzed reaction yields. Controlling the water content for lipase-catalyzed Henry reactions in a DES system is therefore important.

Optimization of the Reaction Parameters for Lipase AS-Catalyzed Henry Reaction in DES–Water Mixture

The lipase AS catalytic system in the DES–water mixture gave the best results. Hence, the reaction was examined further to determine the optimum reaction conditions.

First, lipase AS-catalyzed Henry reactions between 4-nitrobenzaldehyde and nitromethane in ChCl:Gly (1:2) with 30 vol% water were investigated at temperatures ranging from 20°C to 30°C. Fig. 4 illustrates the effects of temperature on the time-course yields. The time-course experiments showed that the total reaction yields of both DES- and lipase-catalyzed reactions increased with the increase in temperature and reaction time. The reaction rate was faster when the reaction temperature was set at 30°C than that at 25°C and 20°C, and a high lipase AS-catalyzed total yield of 95% was achieved in 3 h. However, the DES-catalyzed reaction total yield also reached 20.2%, which was greater than that at 20°C and 25°C, leading to a lipase AS-catalyzed final yield of only 74.8%. This phenomenon could be explained by the lower viscosity of DES at high temperatures, thus minimizing mass transfer issues. When the reaction was carried out at 20°C, a low level of DES-catalyzed reaction was observed; however, the reaction needed at least 10 h to reach a final yield of 83.2%. Thus, 25°C was selected as the optimum reaction temperature, in which a high final yield of 90% was achieved in only 4 h.

Substrate molar ratios of 4-nitrobenzaldehyde and nitromethane were then optimized, and the results are shown in Fig. 5A. The total yields of DES-catalyzed reactions decreased and the total yields of lipase AS-catalyzed reactions improved greatly with the increasing of the substrate molar ratio from 1:2 to 1:15. Further increase in the substrate molar ratio to 1:20 decreased the total reaction yield of the lipase-catalyzed reaction. A maximum final reaction yield of 92.2% was achieved at a substrate molar ratio of 1:15. The additive amount of lipase AS is an important parameter that needs to be optimized as well. As shown in Fig. 5B, the final reaction yields increased with the increase in the additive amount of lipase AS. A high reaction yield of 91.2% was obtained when 10 mg of the enzyme was added. Further increase in the amount of enzyme to 15 mg did not improve the reaction yield. This result might be caused by the aggregation of the enzyme at high concentrations, reducing the accessibility of enzyme

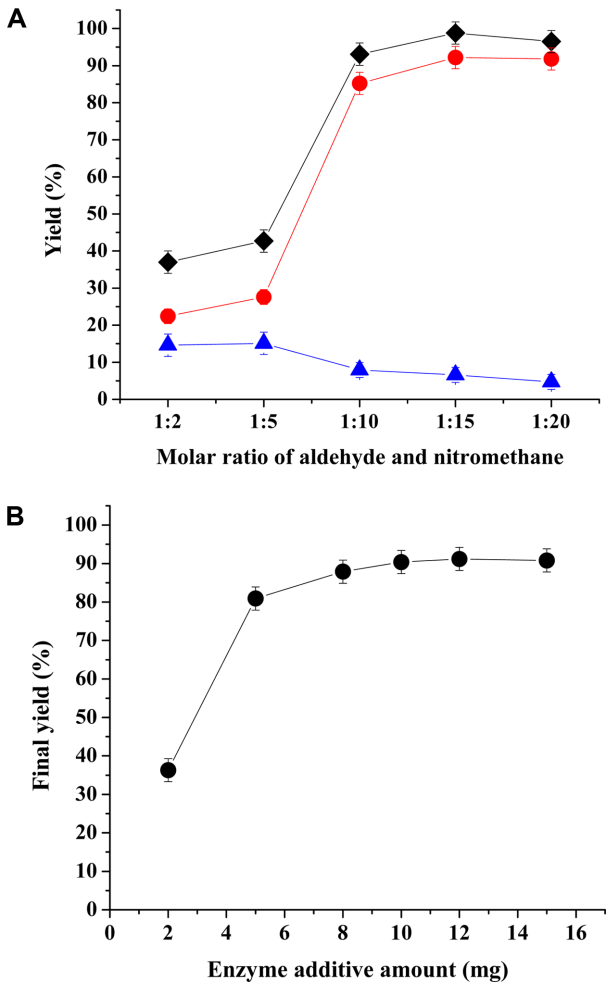


Fig. 5. Optimization of the substrate molar ratio and lipase AS amount.

(**A**) Effects of substrate molar ratios of 4-nitrobenzaldehyde and nitromethane on the lipase AS-catalyzed Henry reaction in ChCl:Gly (1:2) with 30 vol% water. ▲ Total yields of Henry reaction without enzyme; ◆ Total yields of Henry reaction with enzyme; ● Final yields of enzyme-catalyzed Henry reaction. (**B**) Effects of enzyme additive amount on the lipase-catalyzed Henry reaction in ChCl:Gly (1:2) with 30 vol% water.

particles to the substrates. The aggregation of the enzyme might be attributed to the high viscosity of DES solvent, preventing the enzyme from dispersing efficiently in the reaction system. Similar results have been observed by other groups [30, 31].

In summary, the lipase AS-catalyzed Henry reaction achieved a 92.2% final reaction yield under the following optimized reaction conditions: reaction temperature of 25°C, substrate molar ratio of 1:15, enzyme amount of 10 mg, and reaction time of 4 h.

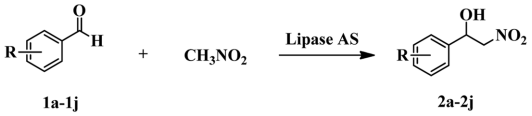
Lipase AS-Catalyzed Henry Reaction of Nitromethane with Other Aromatic Aldehydes

Under the optimized conditions, other aromatic aldehydes were used in the lipase AS-catalyzed Henry reaction to show the generality and expand the scope of this methodology. The results in Table 2 indicate that the reaction system was sensitive to electronic effects, and the benzaldehydes with electron-withdrawing substituents can effectively participate in the reaction. In general, nitro-substituted benzaldehydes furnished nitro alcohol with final reaction yields well above 87.7%. Notably, 4-nitrobenzaldehyde and 2, 4-dinitrobenzaldehyde produced 92.2% and 91.7% final yields, respectively. This result may be because electron-withdrawing groups can enhance the electrophilicity of the carbonyl carbon in the aldehydes, facilitating the reaction. The reaction yields were lower with halo-substituted benzaldehydes than those with nitro-substituted benzaldehydes, which further confirmed that the strong electron-withdrawing groups on the benzene ring had beneficial effects on the Henry reaction. Lipase AS-catalyzed Henry reactions using benzaldehydes with electron-withdrawing group as substrate in the presence of ChCl:Gly (1:2) containing 30 vol% water were superior to that in pure water. In addition, the reaction rates could be greatly improved to provide the corresponding products with satisfactory yields in a short reaction time (4 h). By contrast, aromatic aldehydes containing an electron-donating group gave relatively low yields. When *para*-methoxy-substituted benzaldehyde was used as the substrate, the final reaction yield was only 9.6%. Moreover, no product was created when *para*-methyl-substituted benzaldehyde was used as the substrate, because electron-donating groups render carbonyl carbons in aldehydes less electrophilic. Similar to the benzaldehydes bearing an electron-donating group, no product was found when benzaldehyde with no substituent was used as the reaction substrate when either DES–water or pure water was used as the reaction medium, although it contains relatively small steric hindrance of alkyls.

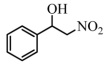
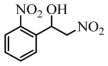
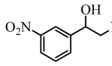
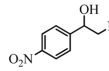
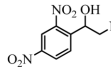
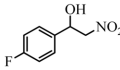
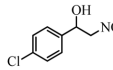
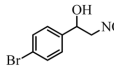
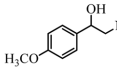
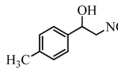
Extension of Methodology to Lipase AS-Catalyzed Aza-Henry Reaction

Given the development of the Henry reactions catalyzed by lipase in DES–water, we further extended the methodology to the aza-Henry reaction to provide a powerful and an efficient method for the synthesis of β-nitro amines, which can be readily converted into 1,2-diamines and α-amino acids. Such molecules are important building blocks in most natural product syntheses [15]. Various catalysts,

Table 2. Lipase AS-catalyzed Henry reaction of nitromethane with other aromatic aldehydes[a].



1a-1j 2a-2j

Entry	Aldehydes	Product	H_2O as solvent		ChCl: Gly (1:2) with 30 vol% water as solvent	
			Time (h)	Yield (%)[b]	Time (h)	Yield (%)[b]
1	 1a	 2a	120	N.R.	48	N.R.
2	 1b	 2b	10	81	4	87.7
3	 1c	 2c	10	85	4	88.5
4	 1d	 2d	10	87	4	92.2
5	 1e	 2e	10	70	4	91.7
6	 1f	 2f	120	46[c]	48	12.3
7	 1g	 2g	120	80[c]	24	62
8	 1h	 2h	120	91	24	89
9	 1i	 2i	120	37[c]	48	9.6
10	 1j	 2j	120	14[c]	48	N.R.

[a]*Reaction conditions*: lipase AS (10 mg), aldehydes (0.15 mmol), nitromethane (2.25 mmol), and solvents (0.7 ml) were stirred at 25°C. N.R. means no reaction.

[b]Final yields of enzyme-catalyzed Henry reaction.

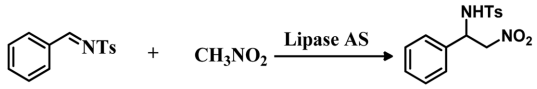
[c]The reaction was performed at 37°C.

including metal complexes and organocatalysts, have been developed for the aza-Henry reaction [11, 21, 32]. However, no reports have focused on enzyme-catalyzed aza-Henry reactions, especially when *N*-sulfonyl-protected imines were used as the substrates.

In this study, the aza-Henry reaction of *N*-Ts (Ts = *p*-tosyl) imines coupling with nitromethane to produce the corresponding nitro amines was selected as the model reaction. The results of lipase AS-catalyzed aza-Henry reactions are summarized in Table 3. The lipase-catalyzed aza-Henry reaction was allowed to proceed first in pure water, but the reaction did not initiate at all. The reaction rapidly progressed when DES was added, indicating that DES is indeed important for the reaction to progress, even though the reaction rate was slow. Similar to the lipase AS-catalyzed Henry reaction in DES, the aza-Henry reaction occurred in ChCl:Gly (1:2) without any catalyst, and a reaction yield of 17.1% was reached. Based on the control experiments, the final reaction yield of 15.8% for the lipase-catalyzed aza-Henry reaction was obtained. Addition of water in ChCl:Gly (1:2) affected the reaction in the same way, evidently decreasing the DES-catalyzed transformation and accelerating the lipase AS-catalyzed reaction. A high final reaction yield of 38.7% was obtained at 30 vol% water content in ChCl:Gly (1:2). Although a longer reaction time (40 h) was required and lower yields were obtained compared with that of the lipase AS-catalyzed Henry reaction, the results are still remarkable. This study is the first to investigate the lipase-catalyzed aza-Henry reaction in DES. Further optimization work is in progress.

In summary, we have developed a simple, environmentally friendly, and efficient catalytic system by using lipase AS

Table 3. Lipase AS-catalyzed aza-Henry reaction of *N*-Ts imines coupling with nitromethane in ChCl:Gly (1:2) with different water content.[a]



Water content (vol%)	Time (h)	Yield (%)[b]	Yield (%)[c]	Yield (%)[d]
0	40	17.1	32.9	15.8
10	40	18.5	40.1	21.6
20	40	12.4	47.9	35.5
30	40	5.2	43.9	38.7
40	40	3.9	41.9	38.0
50	40	3.2	40.8	37.6
100	40	N.R.	N.R.	N.R.

[a]*Reaction conditions*: Lipase AS (10 mg), *N*-Ts imines (0.15 mmol), nitromethane (2.3 mmol), and DES solvent (0.7 ml) were stirred at 25°C. N.R. means no reaction.

[b]Total yields of aza-Henry reaction without enzyme.

[c]Total yields of aza-Henry reaction with enzyme.

[d]Final yields of enzyme-catalyzed aza-Henry reaction.

in DES–water mixtures for the synthesis of nitro alcohol compounds. In this study, DES was first introduced in the lipase-catalyzed Henry reaction. Addition of a certain amount of water to DES remarkably improved the enzyme activity, and the reaction time was greatly shortened. Fluorescence analysis suggested that the improvement of the reaction rate of the lipase AS-catalyzed Henry reaction was mainly caused by the synergistic action of DES and water due to the extensive hydrogen bond network throughout the solvent. By optimizing the reaction parameters, an excellent yield of 92.2% was achieved in a very short reaction time of 4 h. The methodology was also extended to the aza-Henry reaction. Although enantioselectivity for Henry and aza-Henry reactions was not found, we deemed that enantioselectivity can be improved by site-directed mutagenesis or variation of the reaction media. To the best of our knowledge, this study is the first to report on a biocatalyst that can effectively catalyze Henry and aza-Henry reactions in DES.

Acknowledgments

The authors are grateful for the financial support provided by the National Natural Science Foundation of China (Nos. 21372098 and 20802025), Jilin Provincial Science and Technology Sustentation Program (Nos. 201215033 and 20110436), Basic Operating Expenses, and 985 Project at Jilin University.

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