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ORIGINAL PAPER

Overexpression of a recombinant amidase in a complex auto-inducing culture: purification, biochemical characterization, and regio- and stereoselectivity

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Abstract *Rhodococcus erythropolis* AJ270 metabolizes a wide range of nitriles via the two-step nitrile hydratase/amidase pathway. In this study, an amidase gene from *R. erythropolis* AJ270 was cloned and expressed in *Escherichia coli* BL21 (DE3). The activity reached the highest level of 22.04 U/ml in a complex auto-inducing medium using a simplified process of fermentation operation. The recombinant amidase was purified to more than 95% from the crude lysate using Ni-NTA affinity chromatography and Superose S10-300 gel filtration. The V_{max} and K_m values of the purified enzyme with acetamide (50 mM) were 6.89 μmol/min/mg protein and 4.12 mM, respectively, which are similar to those of the enzyme from the wild-type cell. The enzyme converted racemic α-substituted amides, *O*-benzylated β-hydroxy amides, and *N*-benzylated β-amino amides to the corresponding (*S*)-acids with remarkably high enantioselectivity. The ionic liquid [BMIm][PF_6] (1-butyl-3-methylimidazolium hexafluorophosphate) enhanced the activity by 1.5-fold compared with water. The adequate expression of the enzyme and excellent enantioselectivity of the recombinant amidase to a broad spectrum of amides suggest that the enzyme has prospective industrial-scale practical applications in pharmaceutical chemistry.

Keywords Amidase · *Rhodococcus erythropolis* AJ270 · Stereoselectivity · Ionic liquid · Complex auto-inducing medium

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Introduction

Amidase (EC 3.5.1.4) catalyzes the hydrolysis of amides to the corresponding acids and ammonia. Amidases have been the subject of much research interest because a number of carboxylic acids and their amide derivatives are important intermediate compounds for industrial or pharmaceutical use. To date, more than 26 microbial amidases have been purified and characterized [31]. These amidases can be classified into two types according to their substrate specificities [10]. One category includes aliphatic amidases that only hydrolyze short-chain aliphatic amides; the other, amidases that hydrolyze mid-chain amides, arylamides, and heterocyclicamides. Most bacteria that have the second type of amidases also contain nitrile hydratases. Nitriles are successively transformed by nitrile hydratase and amidase into the corresponding amides and carboxylic acid derivatives. *Rhodococcus erythropolis* AJ270, isolated from a disused industrial site on the banks of the River Tyne in

northeast England [1, 2], hydrates numerous nitriles to the corresponding amides and carboxylic acids with high regio- and stereoselectivity [23, 35, 41, 42]. Although nitrile hydratase shows enantioselectivity with some substrates, such enantioselectivity, is largely due to amidase [43]. Previous research has shown that a large number of amidases hydrolyze (*S*)-amides bearing an α-stereocenter with high enantioselectivity [14, 16, 18, 45] and some with (*R*)-enantioselectivity [4, 25, 29, 30]. As for regioselectivity, most amidases recognize an α-stereocenter with high stereoselectivity. The case of L-carnitine amidase is a rare example of stereoselectivity toward a *β*-substituted amide [15]. One study showed that under mild conditions, whole cells of *R. erythropolis* AJ270 transformed α-substituted racemic amides with high (*S*)-enantioselectivity into the corresponding enantiopure acids, but the enantiocontrol of the reaction was very disappointing when performed on racemic *β*-hydroxy and *β*-amino alkanenitriles [24].

Amidases from bacteria other than *R. erythropolis* have been cloned and expressed [12, 17, 33, 39]. However, most of these expression systems have shown low efficiency, and the production of enzymes has been inadequate for industrial use. In the current study, the gene encoding amidase in *R. erythropolis* AJ270 was expressed at high levels in a complex auto-inducing (CAI) medium. The recombinant amidase was also able to transform α-, *β*-benzyloxy-, and *β*-benzylamino-substituted amides into their corresponding amides and acid derivatives with high enantioselectivity. The activities of recombinant amidase in two kinds of ionic liquids, [BMIm][PF_6] (1-butyl-3-methylimidazolium hexafluorophosphate) and [BMIm][BF_4] (1-butyl-3-methylimidazolium tetrafluoroborate), were also investigated.

Materials and methods

Plasmids, strains, and chemical reagents

The expression vector pET22b was preserved in our laboratory. pEASY T1 Simple Vector, *E. coli* strain DH5α, and *E. coli* BL21 (DE3) were purchased from Transgen (China). *R. erythropolis* AJ270 was preserved in our laboratory. (It was originally isolated from the banks of the River Tyne, England.) Restriction enzymes were purchased from Takara, Ltd. (Japan). Racemic amides (2-ethyl-phenyl-acetamide, 2-methyloxyphenylacetamide, 3-hydroxy-3-phenyl-propionamide, 3-amino-3-phenyl-propionamide, 3-benzylamino-3-phenyl-propionamide, 3-benzyloxy-valeramide, and 3-allyloxy-butyramide) were synthesized by the Institute of Chemistry at the Chinese Academy of Sciences. The ionic liquids [BMIm][BF_4] and [BMIm][PF_6] were synthesized according to the methods of Lu and Cai [5, 21]. Acetamide and other chemicals were commercial products of the highest grade obtainable.

DNA isolation

R. erythropolis AJ270 was grown in Luria–Bertani (LB) broth containing 50 mmol/l acetamide for 2 days. Total DNA was isolated using a Genomic DNA Isolation Kit (Transgen) according to the manufacturer's instructions.

Cloning and expression of the amidase gene in *E. coli*

The amidase gene of *R. erythropolis* AJ270 was amplified using the primers *Ami*-F (5′-GGAATTCCATATGGCGAC AATCCGACC-3′) and *Ami*-R (5′-ATAATAATGCGGCC GCGGCGGGGCTGAGTTGTGG-3′), with *Nde*I and *Not*I sites (*underlined*) added to the forward and reverse primers, respectively. The PCR products were subcloned into the pEASY T1 Simple Vector for sequence analysis. The expression plasmid of amidase, PAmi, was constructed by subcloning the *Nde*I/*Not*I fragment into the corresponding site of pET22b. PAmi was then transformed into the host *E. coli* BL21 (DE3). The protein was expressed as a C-terminal fusion to the His_6 purification tag.

The seed culture (5 ml) of *E. coli* BL21 (DE3) bearing plasmid PAmi was prepared by growing cells on a rotary shaker (200 rpm) for approximately 15 h at 37°C. It was then inoculated into an LB medium containing ampicillin (100 μg/ml), which was subsequently cultured at 37°C and 200 rpm until the OD_{600} reached approximately 0.8. Isopropyl *β*-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM to induce the expression of the enzyme. The cultures were incubated at 25°C for 4 h at 200 rpm.

Culture optimization

A medium of high-density culture (MHDC) and CAI medium were developed to increase the production of the recombinant amidase and simplify the fermentation process. The recipe for CAI medium was based on the method described by Studier [37], with slight modifications, and the effects of some components in the cultures were then tested. Overnight seed cultures of MHDC were inoculated into CAI medium at a ratio of 1:100 and incubated at different temperatures for 17 h.

Purification of recombinant amidase

The cells were harvested by centrifugation, suspended in ice-cold lysis buffer [50 mM Tris-HCl pH 8.0, 0.3 M NaCl, 10 mM imidazole, and 1 mM DL-dithiothreitol (DTT)] and disrupted by sonication. The supernatant was

collected by centrifugation. The amidase was purified from the lysate by a combination of Ni-NTA affinity chromatography and Superose S10-300 gel filtration. The homogeneity of the purified enzyme was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and native PAGE using 12% acrylamide gel. Protein bands were visualized by Coomassie brilliant blue R250 staining.

Amidase activity assays and protein determination

Amidase activity was determined by the release of ammonia from the hydrolysis of amides. Each 800-μl reaction system contained 10 μl of diluted enzyme sample, 100 mM phosphate buffer (pH 7.5), and 50 mM substrate. Phosphate buffer was used as a blank. The substrate blank was conducted without the addition of substrate. The reaction mixtures were incubated at 37°C and 200 rpm for 15 min. Two hundred microliters of 1 M HCl was added to stop the reaction. Ammonia concentration was determined using the nesslerization method [35]. One unit of activity was measured by the formation of 1 μmol/min at pH 7.5 and 37°C. The specific activity of the amidase was expressed in units (U) per milligram of protein. Protein concentrations were determined using the Bradford method [3], and bovine serum albumin (Promega, USA) was used as the standard protein.

Kinetic determination

The K_m and V_{max} for acetamide, propionamide, acrylamide, and benzamide were determined with concentrations of 0.5, 1, 2, 4, 8, 16, and 32 mM in the reaction system. The K_m and V_{max} were determined using a direct linear plot [34].

Regio- and stereoselectivity of amidase

Several racemic amides were used to study the regio- and stereoselectivity of the recombinant enzyme including α-substituent amides (2-ethyl-phenylacetamide and 2-methyloxy-phenylacetamide), α-unsubstituted/β-substituted amides (3-hydroxy-3-phenyl-propionamide, 3-amino-3-phenyl-propionamide, 3-benzylamino-3-phenyl-propionamide, and 3-benzyloxy-valeramide), and an unsaturated side-chain amide (3-allyloxy-butyramide). Fifty milliters of potassium phosphate buffer (0.1 M, pH 7.0) and 1 mM substrate were added to a 150-ml screw-cap Erlenmeyer flask and incubated at 30°C for 0.5 h at 200 rpm. Three hundred micrograms of the recombinant enzyme was added to the flask, and the mixture was further incubated at 30°C and 200 rpm. After a specified period, 500 μl of the reaction mixture was collected and then mixed with 100 μl HCl (1 M) to stop the reaction. Two hundred microliters of isopropyl alcohol was added to resolve the precipitation after a process of ether extraction (700 μl) and blow-drying. Five microliters of the solution was used in the high-performance liquid chromatography (HPLC) assay as previously reported [23, 24, 44]. All products were characterized by their spectral data. Enantiomeric excess values (ee%) were obtained via HPLC analysis using a chiral stationary-phase column.

Activities of amidase in ionic liquids

Each 800-μl reaction system contained 10 μl of purified enzyme, 80 μl of 500 mM substrate, 10–90% of the total volume of various ionic liquids, and 100 mM phosphate buffer, pH 7.5. Other parameters of the reaction were the same as before. Ammonia was determined as described by Fawcett and Scott [9]. The reaction system without ionic liquid was used as the control for 100% activity.

Results

Expression of amidase gene in *E. coli*

The amidase gene from *R. erythropolis* AJ270 was amplified by PCR and subcloned into the pET22b expression vector with a His_6 affinity tag at the C-terminal end. The gene contained a 1,566-bp open reading frame [28]. The recombinant plasmid was transformed into *E. coli* BL21(DE3). Expression of the recombinant amidase in LB medium indicated that the highest activity was 2.54 U/ml culture at an induction temperature of 25°C with 1 mM IPTG. The amidase activity in CAI medium increased to 22.04 U/ml, an increase nearly 8.7 times that in LB medium. Moreover, the introduction of CAI medium greatly simplified the operation and lowered the cost of amidase production. Thus, the optimal conditions of enzyme expression in this study were in CAI medium incubated at 25°C for 17 h at 200 rpm.

Purification of the recombinant amidase

Purification of recombinant amidase was performed via a combination of affinity chromatography and gel filtration chromatography. The recovery was approximately 33.8%, with a total purification factor of 6.9 (Fig. 1a, Table 1). Native PAGE analysis showed that a single band lies at the site at 110 kDa (Fig. 1b), suggesting that the enzyme was a homodimer corresponding to the calculated molecular mass of 55,528 Da, based on the gene sequence of the recombinant amidase.

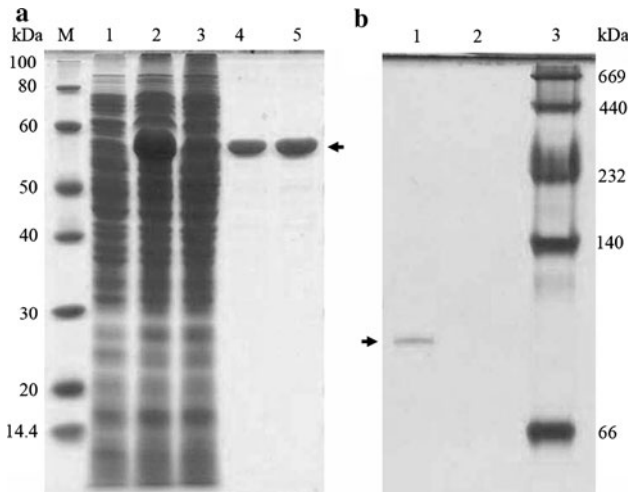


Fig. 1 Electrophoresis analysis of recombinant amidase. **a** SDS-PAGE analysis of recombinant amidase from *E. coli* BL21/PAmi at different purification steps. *M* protein marker (from *top*: 100, 80, 60, 50, 40, 30, 20, and 14.4 kDa); *lane 1* supernatant of the lysate of uninduced culture; *lane 2* supernatant of the lysate of culture induced at 25°C; *lane 3* flowthrough on affinity column; *lane 4* purified amidase from affinity column; *lane 5* purified amidase from Superpose S10-300. *Arrows* point to the purified protein. **b** Native PAGE (12%) analysis of purified recombinant amidase from *E. coli.* BL21/PAmi. *Lane 1* purified recombinant amidase; *lane 2* empty; *lane 3* HMW native protein marker (GE Healthcare, USA; from *top*: thyroglobulin 669 kDa, ferritin 440 kDa, catalase 232 kDa, lactate dehydrogenase 140 kDa, and albumin 66 kDa). *Arrows* point to the purified protein

Kinetic parameters of the recombinant amidase

The kinetic parameters of the recombinant amidase for the amide compounds were calculated (Table 2). The K_m values for acetamide, propionamide, acrylamide, and benzamide were 4.12 ± 1.36, 6.32 ± 1.55, 16.72 ± 6.72, and 1.02 ± 0.07 mM, respectively, indicating that benzamide is the most preferable substrate. The V_{max} of benzamide was 47.67 μmol/min/mg protein. Acrylamide was not significantly hydrolyzed. These results are similar to those obtained for the amidase from *Rhodococcus sp*. R312 [11].

Effects of pH and temperature on the enzyme activity

The optimal temperature and pH level for enzyme activity were 40°C and 7.5, respectively. After 42 days in 50 mM potassium phosphate (pH 7.5) at 4°C, the residual activity of the recombinant enzyme was approximately 72% of its initial activity. Only 49% of residual activity was found after incubation at 37°C for 4 days.

Table 2 Kinetic parameters of recombinant amidase with various substrates

Compound	K_m (mM)	V_{max} (μmol/min/mg protein)
Acetamide	4.1 ± 1.36	6.9 ± 1.05
Propionamide	6.3 ± 1.55	30.8 ± 5.38
Acrylamide	16.7 ± 6.72	0.6 ± 0.02
Benzamide	1.0 ± 0.07	47.7 ± 7.67

Regio- and enantioselectivity of the recombinant amidase

Two racemic α-substituent amides, **1a** and **1b** (Table 3), four racemic α-unsubstituted/β-substituted amides, **1c–f** (Table 4), and an unsaturated side-chain amide, **1g** (Table 5), were used to determine the regio- and enantioselectivity of the recombinant amidase in amide hydrolysis. The results demonstrated that the recombinant amidase can hydrolyze penylacetamides bearing a variety of substituents at the α-position with strict (*S*)-enantioselectivity. Optically pure (*R*)-amides and (*S*)-acids were then produced. However, the reaction was strongly affected by the nature of the α-substituent. Biotransformation was efficient when the substituent was a hydrophobic group, such as ethyl. However, the rate of hydration slowed down when the substituent was a polar moiety, such as a methoxy group. When acting on 3-hydroxy-3-phenyl-propionamide and 3-amino-3-phenyl-propionamide, the enzyme yielded good chemical productivity but very low enantioselectivity (<30%). On the other hand, enantioselectivity remarkably increased with the *O*-benzylated and *N*-benzylated substrates (enantiomeric excess up to 99%) with relatively low catalytic capability. The enzyme also acted on the unsaturated side-chain amide with low enantioselectivity.

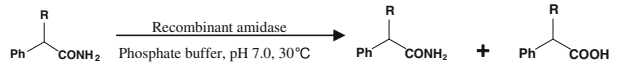
Effects of ionic liquid on enzyme activity

[BMIm][BF_4] and [BMIm][PF_6] were added into the reaction system, and the activities were then detected

Table 1 Purification of recombinant amidase expressed in *E. coli* BL21 (DE3) bearing plasmid PAmi

Step	Volume (ml)	Total activity (U)	Total protein (mg)	Specific activity (U/mg protein)	Purification factor (fold)	Recovery (%)
Crude enzyme	42	2,468.2	666.1	3.7	1.0	100
Ni-NTA	16	876.5	38.3	22.9	6.2	35.5
Superose S10-300	10	835.4	32.6	25.6	6.9	33.8

Table 3 Enantioselective biotransformations of racemic α-substituent amides



(+/-)-1(a-b) *R*-(+)-2(a-b) *S*-(-)-3(a-b)

Entry	(±)-**1**	Time (h)	**2** (yield%)[a] (ee%)[b]	**3** (yield%)[a] (ee%)[b]
1	**1a** R = Et	17	49.8 (>99)	49.2 (>99)
2	**1b** R = OMe	168	49.4 (5.9)	6.77 (>99)

[a] Isolated yield

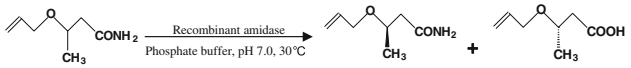
[b] Determined by chiral HPLC analysis

(Fig. 2). The activity increased when the reaction system contained [BMIm][PF_6]. A concentration of 50% [BMIm][PF_6] in the reaction system gave the highest yield, which was 1.5-fold that of the activity in the aqueous reaction system. However, [BMIm][BF_4] showed an inhibitory effect on enzymatic activity. The K_m and V_{max} of the enzymes with acetamide, propionamide, acrylamide, and benzamide were determined for the [BMIm][PF_6]-containing reaction system. The results were similar to those obtained in the aqueous reaction system (data not shown).

Discussion

The amidase gene from the species *R. erythropolis* AJ270 was cloned, heterologously expressed at a high level in *E. coli* BL21(DE3), and purified to homology via affinity chromatography and gel filtration. MHDC was introduced, and the activity of the culture successfully increased by approximately three times that in the LB medium to increase soluble protein expression. MHDC was further

Table 5 Enantioselective biotransformations of racemic 3-allyloxybutyramide



(+/-)-1g *R*-(+)-2g *S*-(-)-3g

Entry	(±)-**1**	Time (h)	**2** (yield%)[a] (ee%)[b]	**3** (yield%)[a] (ee%)[b]
7	**1g**	8	52.9 (14.2)	15.4 (88.7)
		24	42.9 (31.5)	27.1 (90.0)
		72	29.8 (87.9)	44.9 (91.5)

[a] Isolated yield

[b] Determined by chiral HPLC analysis

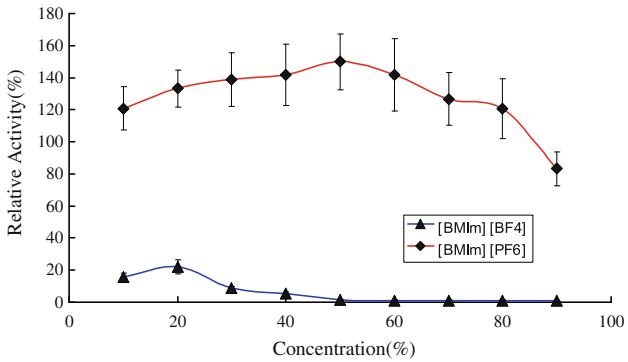
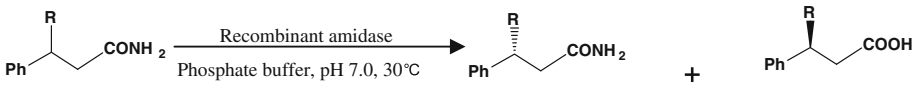


Fig. 2 Activity of the recombinant amidase in ionic liquid-containing reaction systems. The activity of the recombinant amidase increased in a 50% [BMIm][PF_6]-containing reaction system and reached a maximum of 150% of that in water. [BMIm][BF_4] inhibited recombinant amidase activity dramatically, with no more than 22% activity compared to water

Table 4 Enantioselective biotransformations of racemic β-substituent amides



(+/-)-1(c-f) *R*-(+)-2(c-f) *S*-(-)-3(c-f)

Entry	(±)-**1**	Time (h)	**2** (yield%)[a] (ee%)[b]	**3** (yield%)[a] (ee%)[b]
3	**1c** R = OH	1	18.9 (10.4)	39.8 (7.7)
		2	–[c]	49.7 (3.8)
4	**1d** R = NH_2	1	28.5 (29.6)	30.3 (4.7)
		2	–[c]	50.6 (16.7)
5	**1e** R = OBn	72	44.9 (61.9)	16.3 (87.2)
		240	46.3 (88.4)	48.4 (94.2)
6	**1f** R = NBn	120	47.6 (93.5)	42.6 (>99)

[a] Isolated yield

[b] Determined by chiral HPLC analysis

[c] Not detectable

optimized by the addition of lactose and supplemented with sodium succinate and sodium citrate. The concentration of tryptone and yeast extract were doubled, and CAI medium was obtained. The activity of expressed amidase increased to 22.04 U/ml culture with the specific activity of 3.71 U/mg protein, which is significantly higher than previously reported. This is comparable with the results reported for *R. erythropolis* MP50 amidase (0.69 U/mg; phenylacetamide) [39], *Microbacterium* sp. AJ115 amidase (2.81 U/mg; acetamide) [7], *R. erythropolis* No. 7 (0.97 U/mg; isobutyramide) [31], and *O. anthropi* NCIMB 40321 (2 U/mg; L-Val-NH_2) [36]; it is considerably greater than that of the *R. erythropolis* AJ270 wild-type cell (1.3 U/mg; acetamide) (data from our experiment). The high level of expression of recombinant protein in CAI medium and its high maneuverability make it suitable for a wide range of industrial applications.

The optimal temperature of the recombinant amidase is 40°C, with acetamide as the substrate, which is lower than the optimal temperatures of the amidases from wild-type *R. erythropolis* MP50 [14], *Rhodococcus* sp. R312 [11], and *R. erythropolis* No.7 [31], whose optimal temperatures are approximately 50°C. The recombinant amidase has a lower reaction temperature, neutral pH, and good thermal stability.

The enantio- and stereoselectivity of the amidases have been studied for production of optically active D- or L-amino acids [13] and for enantioselective synthesis of (*R*)- or (*S*)-acids as intermediates of pharmaceuticals or herbicides. Studies of biotransformations using *R. erythropolis* AJ270 whole cells have indicated that the nitrile-degrading enzymes in the cells have high enantioselectivity [24, 35, 42, 44]. In this two-enzyme system, enantioselectivity is largely due to the amidase because nitrile hydratase shows very low enantioselectivity with some substrates [43]. Research has shown that biotransformation of nitriles by whole cells is an effective way of obtaining enantiopure amides as well as chiral carboxylic acids and their derivatives bearing an α-stereocenter. Optically active β-hydroxy and β-amino acids and their derivatives are key intermediates in the synthesis of natural products and biological compounds, but the existing amidase has low enantioselectivity to β-hydroxy and β-amino amides, resulting in much difficulty with obtaining those active entries by biotransformation. Site-directed mutagenesis and directed evolution to modify the structure of the enzyme have been chosen to solve this problem, but these methods are complex, costly, and time consuming. Substrate engineering, as an alternative method, seems to be useful in improving the efficiency and selectivity of biocatalysis. Ma et al. [22–24] found that using the *O*-benzyl and *N*-benzyl protection/docking method dramatically enhanced the enantioselectivity of biotransformations. The results of the current study indicate that, compared with free β-hydroxy and β-amino phenylpropionamides, the enzyme that catalyzed the hydrolysis of *O*-benzyl and *N*-benzyl phenylpropionamides afforded remarkably high enantioselective biotransformations. The remote chiral recognition mechanism, which asserts that the chiral recognition site of the enzyme may be located far from the catalytic center, can explain this phenomenon [23]. Considering the easy protection and deprotection operations, this enzyme provides good resolution for the biosynthesis of compounds with free β-hydroxy and β-amino amides and acids, as well as their derivatives.

Ionic liquids are low-melting (<100°C) salts, which represent a new class of nonmolecular ionic solvents. They are characterized by their negligible vapor pressure, recyclability, as well as excellent chemical and thermal stability; they are eco-friendly and can be dissolved in a wide variety of organic and inorganic chemicals. Owing to these attributes, numerous studies have used ionic liquids as alternative solvents in enzyme catalysis [32, 40, 46]. Compared with conventional solvents, the activity, stability, and selectivity of enzymes in ionic liquids are more favorable [6, 19, 20, 27]. The chemical and physical properties of ionic liquids can be modified by changing the cation, anion, or both. Enzymes in ionic liquids have been reported to follow similar catalytic mechanisms as in water or organic solvents. In this study, [BMIm][BF_4] and [BMIm][PF_6] were tested. The results showed that the enzyme with [BMIm][PF_6] had a dramatically higher activity than that with [BMIm][BF_4]. Although both ionic liquids have similar polarity, viscosity, and oxygen solubility, their hydrophobicities are different. Because of the highly hydrophilic nature of [BMIm][BF_4], it can enter aqueous microenvironments surrounding the enzyme molecules and directly interact with the enzyme to inactivate it. In addition, the polarity of the B–F bond in BF_4^- should be slightly higher than that of the P–F bond in PF_6^-, according to the electronegativity theory [26]. This may result in a more disruptive interaction with the enzyme and may therefore be partially responsible for the lower enzyme activity in [BMIm][BF_4]. Moreover, amidase showed very low activity in hydrophilic organic solvents, such as acetone, ethyl acetate, and isopropanol in the current study (data not shown).

The recombinant amidase followed classic Michaelis–Menten kinetics in the reaction system containing 50% [BMIm][PF_6], as it did in water. The K_m values of the enzymes with acetamide, propionamide, acrylamide, and benzamide were similar to those in the aqueous system. The enhanced activity may be attributed to the higher stability of the enzyme structure, because enzymes can be stabilized by kosmotropic anions such as PF_6^- [38]. Kosmotropic anions can take up water molecules associated

with the enzyme and reduce the average free energy of the bulk water [8]. Thus, the hydrophobic effect drives the enzyme to keep its native formation, rendering the enzyme more stable. This state is also catalytically more active, thus yielding a higher V_{max} [47].

Conclusion

The amidase gene of *R. erythropolis* AJ270 was cloned and expressed at a high level (22.04 U/ml) in *E. coli* as a result of the introduction of the CAI medium. The purified enzyme hydrolyzed a variety of amides with a broad spectrum, including short-chain aliphatic amides, mid-chain aliphatic amides, and arylamides. The enzyme transformed amides bearing α- or β-stereocenters with *O*-benzyl and *N*-benzyl protection with high enantioselectivity. The enzyme activity increased by 1.5-fold when the reaction system contained 50% of [BMIM][PF_6]. This study lays the groundwork for further research to improve biotransformations affected by ionic liquids.

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