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Asymmetric reductive resolution of racemic sulfoxides by recombinant methionine sulfoxide reductase from a *pseudomonas monteilii* strain

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ABSTRACT

In this study, the methionine sulfoxide reductase A from a *pseudomonas monteilii* strain (*pm*MsrA) was reported to synthesize optically active sulfoxides (*R*)-**1a**-**4a**, through asymmetric biocatalytic reductive resolution. Several biotransformation parameters including the reaction time, cell density, and substrate concentration were optimized. Moreover, Substrate scope of *pmMsrA* catalyzed asymmetric reductive resolution was investigated, which gave chiral (*R*)-**1a**-**4a** with 61%-97% *ee*.

1. Introduction

Chiral sulfoxides are kinds of valuable organosulfur compounds for synthesis of chemical materials, pharmaceuticals and agrochemicals, in which the sulfoxide group usually used as key building blocks [1–3]. From the view point of intended side-effects and pharmacological activities, pharmaceutical compounds having an asymmetric center in their molecules are usually used as their optically active form instead of racemic form [3]. Therefore, it is desired to develop novel and efficient methods for the synthesis of optically active sulfoxides.

In the last few years, different methodologies for the synthesis of enantiopure sulfoxides have been developed. Optically active sulfoxides are mainly preparaed by asymmetric oxidation of prochiral sulfides [4–7] and asymmetric resolution of racemic sulfoxides [8,9]. Although plenty of chemical methods for the production of enantio-enriched sulfoxides have been reported [3–5], biocatalytic approaches have become a useful and greener alternative in stereoselective synthesis due to their multiple advantages [10]. To date, most of studies foucused on the asymmetric oxidation of sulfides to chiral sulfoxides by whole-cell systems or isolated enzymes [6,11–14]. Noteworthily, the use of whole-cell systems for the asymmetric oxidative/reductive resolution of racemic sulfoxides for enantiopure sulfoxides preparation are less reported [8]. For example, Xu and co-workers reported a fed-batch reaction of oxidative resolution process to synthesize (*S*)-phenyl methyl sulfoxide with 37.8 mM product concentration in 93.7% *ee* using resting cells of *Rhodococcussp*. ECU0066 [8]. However, only very few reports have focused on the enantioselective reduction of racemic sulfoxides catalyzed by baterial cells under anaerobic conditions [9,15–17]. For example, Hanlon and co-workers reported an investigation of several different microbial cells growth under anaerobic conditions, indicating that bacterial *S*-oxide reductases could exhibit activity for the reduction of sulfoxides [16]. Recently, Tudorache and colleagues developed a sequential deracemization of sulfoxides via whole-cell resolution conbined with heterogeneous Ta_2O_5–SiO_2 oxidation protocol, in which a 56% yield of (*S*)-methyl tolyl sulfoxide in 97.5% *ee* was obtained after 3 deracemization cycles in 1.0 g/L substrate concentration [9]. Though these research works represent alternatives for the synthesis of optical active sulfoxides, using biocatalytic reductive resolution of racemic sulfoxides for chiral sulfoxides preparation has not yet been well investigated.

In our previous research, a bacterial strain named *Pseudomonas monteilii* CCTCC M2013683 was reported to synthesize chiral sulfoxides with 99% *ee* [7]. After transcriptional analysis of this bacterium [18], a gene encoding methionine sulfoxide reductase A (MsrA) was cloned in this study. As we know, methionine sulfoxide reductases (Msrs) are important protein repair enzymes that catalyze the reduction of methionine sulfoxide to methionine [19–21]. These enzymes play a pivotal defensive role against oxidative stress from bacteria to human [22–24]. MsrA is specific to the (*S*)-enantiomer of methionine sulfoxide and has a protective role against oxidative stress [25]. These reports implied that the MsrA has the potential application in reductive resolution of *rac*-sulfoxides to prepare chiral sulfoxides with *R* configuration. Thus, in this research, the MsrA from *Pseudomonas monteilii* CCTCC M2013683 (*pm*MsrA) was recombinant expressed and used for preparation of optically active sulfoxides by biocatalytic reductive resolution approach.

2. Material and methods

2.1. Chemicals, plasmisd and microbial strains

Chemical reagents such as methyl phenyl sulfoxide **1a** (>98%), methyl phenyl sulfide **1b** (>99%) were commercially available with purity of analytic grade or chromatographic grade. *Rac*-**2a-4a** were synthesized according to literature [26]. Plasmid pET-28a was purchased from Novagen (Merck KGaA). Competent cell of *E. coli* DH5α

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and BL21 (DE3) were purchased from Tiangen (Beijing, China). Microorganism *Pseudomonas monteilii* CCTCC M2013683 was isolated and identified by our laboratory.

2.2. Gene cloning and recombinant expression of pm*MsrA*

The cDNA of *Pseudomonas monteilii* CCTCC M2013683 was prepared according to literature [18] and used as the PCR templates to amplify *pmMsrA* gene. The primer sequences for gene cloning were 5′-AACCGGATCCATGGTCCTGCGTTCGGAA-ATC-3′ and 5′-AAGGAAGCTTGTTACCCTGCAGGCTCGGTG-3′, with *Bam*H I and *Hin*d III restriction sites underlined, respectively. PCR amplified genes were digested with coresponding restrictive enzymes. The digested DNA fragment was ligated into the multiple cloning site (MCS) of the pET-28a which was also digested with *Bam*H I and *Hin*d III restriction enzymes. Afterwards, the resulting plasmid was transformed into chemical competent *E. coli* DH5α cells and confirmed by PCR and sequencing. To induce the soluble expression of recombinant enzyme, the plasmid was transformed into chemical competent *E. Coli* BL21(DE3) cells. A single colony was inoculated in 3 mL LB medium overnight at 37 °C as the seed culture. One percent seed were transferred in 50 mL LB medium in a 250 mL shaking flask with baffles. Cells were grown at 37 °C to OD_{600} about 0.6 and then induced by adding isopropyl-β-D-thiogalactopyranoside (IPTG) to final concentrations of 0.2–0.5 mM. Cells grown at 20 °C for anothor 2–20 h were harvested and suspended in PBS buffer. After ultrasonic fragmentation, the supernate was subjected to SDS-PAGE gel electrophoresis for analysis of recombinant protein expression.

2.3. Whole-cell biotransformation

Cells after IPTG induction were washed twice with sterile PBS buffer (50 mM, pH 7.5) and resuspended at the cell density of 10–40 gdcw/L in the same buffer. Substrates **1a-4a** were added to the reaction mixture in the final concentration of 2–5 mM. Biotransformation was performed in a 25 mL glass vials with screw caps with 5 mL cell suspensionat 30 °C. After incubation with shaken at 300 rpm for 4–30 h, the reaction mixture was extracted with 10 mL of ethyl acetate by vigorous shaking. The organic layers were separated by centrifugation and then dried by anhydrous sodium sulfate.

2.4. Analytical methods

Conversion of sulfoxides **1a**-**4a** to sulfides **1b-4b** was determined by GC with anagilent 7820 GC (Agilent Technologies, Santa Clara, CA) using a HP-5 column (30 m × 0.32 mm × 0.25 μm) and an FID detector. The 1 mM of DMSO was added to the organic layer as an internal standard for GC analysis. The temperature setting was programmed as follows: T0: 50 °C; dT/dt: 30 °C/min, T1: 130 °C, 1.5 min; dT/dt: 20 °C/min, T2: 135 °C, 0.7 min; dT/dt: 20 °C/min, T3: 180 °C, 2 min; split ratio, 1: 10. Under these conditions, the retention times were 1.90 min for DMSO, 3.06 min for **1b**, 5.04 min for **1a**, 4.705 mim for **2b**, 6.424 min for **2a**, 4.454 min for **3b**, 6.778 min for **3a**, 4.45 min for **4b** and 6.805 min for **4a**. The *ee* values of products were determined using a Shimadzu™ Prominence HPLC on a Daicel™ OD-H chiral column (250 × 4.6 mm, 5 μm) at 25 °C with a flow rate of 1 mL/min and UV detection at 220 nm, and the mobile phase used was 5% isopropanol: 95% *n*-hexane. The configuration was assigned by comparison ratation data with the references [7]. Optical rotation data of sulfoxides was determined by RUDOLPH research analytical Autopol I-AP. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker-300 (300/75 MHz) spectrometer using $CDCl_3$ as a solvent and TMS as an internal standard.

2.5. Spectral and ee data for the biosynthesized compounds

Compound (*R*)-**1a**: $[\alpha]^{25}_D$ = +134.8 (*c* = 0.50, acetone) for (*R*), 93.4% *ee*; lit: $[\alpha]^{25}_D$ = +142.8 (*c* = 0.53, acetone) for (*R*), 99% *ee* [7]. Daicel™OD-H chiral column (250 × 4.6 mm, 5 μm) at 25 °C with a flow rate of 1 mL/min and UV detection at 220 nm. Retention time: 22.6 min for (*R*)-**1a**, 31.5 min for (*S*)-**1a**. ^{1}H NMR (400 MHz, $CDCl_3$): 7.63-7.60 (m, 2H), 7.51-7.46 (m, 3H), 2.69 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 43.9, 123.5, 124.0, 131.09, 145.63.

Compound (*R*)-**2a**: $[\alpha]^{25}_D$ = +234.8 (*c* = 0.50, acetone) for (*R*), 93.2% *ee*; lit: $[\alpha]^{25}_D$ = +269.1 (*c* = 0.66,acetone) for (*R*), 98% *ee* [5]. Daicel™OD-H chiral column (250 × 4.6 mm, 5 μm) at 25 °C with a flow rate of 1 mL/min and UV detection at 220 nm. Retention time: 22.1 min for (*R*)-**2a**, 23.7 min for (*S*)-**2a**.^{1}H NMR (400 MHz, $CDCl_3$): δ2.73 (s,3H), 7.46 (s, 3H), 7.65 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ44.1, 121.7, 123.8, 130.8, 131.3, 135.8, 147.9.

Compound (*R*)-**3a**: $[\alpha]^{25}_D$ = +66.6 (*c* = 0.50, acetone) for (*R*), 61.2% *ee*; lit: $[\alpha]^{25}_D$ = + 98.1 (*c* = 0.60, acetone) for (*R*), 99% *ee* [5]; Daicel™OD-H chiral column (250 × 4.6 mm, 5 μm) at 25 °C with a flow rate of 1 mL/min and UV detection at 220 nm. Retention time: 17.2 min for (*R*)-**3a**, 18.3 min for (*S*)-**3a**. ^{1}H NMR (400 MHz, $CDCl_3$): δ2.81 (s,3H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.43 (t, *J* = 6.0 Hz, 1H), 7.52 (t, *J* = 6.0 Hz, 1H), 7.94 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ41.7, 125.4, 128.3, 129.9, 132.1.

Compound (*R*)-**4a**: $[\alpha]^{25}_D$ = +118.5(*c* = 0.50, acetone) for (*R*), 97.0% *ee*; lit: $[\alpha]^{25}_D$ = +115.8 (*c* = 0.73, acetone)for (*R*), 97% *ee* [5]; Daicel™OD-H chiral column (250 × 4.6 mm, 5 μm) at 25 °C with a flow rate of 1 mL/min and UV detection at 220 nm. Retention time: 22.5 min for (*R*)-**4a**, 23.6 min for (*S*)-**4a**. ^{1}H NMR (400 MHz, $CDCl_3$): δ2.71 (s,3H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.58 (d, *J* = 8.0 Hz,2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ44.1, 125.1, 129.7, 131.1, 137.4, 144.3.

3. Results and discussion

3.1. Construction of the whole-cell pm*MsrA* biocatalyst

In our previous study, a bacterial strain named *P. monteilii* CCTCC M2013683 showed the activity for synthesis of chiral sulfoxides with high activity and enantioselectivity [7]. After RNA-seq transpritional analysis of this strain [18], a gene encoding *pm*MsrA was predicted to selectively reduce the (*S*)-methionine sulfoxide to methionine based on KEGG analysis (Fig. 1A). To investigate whether *pm*MsrA could be used for (*R*)-sulfoxides preparation through reductive resolution, we designed the recombinant plasmid of *pm*MsrA based on the prokaryotic expression vector pET-28a (Fig. 1B). Firstly, the 669 bp of nucleic acid fragment was applified by RT-PCR techenique (Fig. 2A) and the gene sequence was submitted to the genebank (accession number: KY171941). After cloning of *pmMsrA* to the pET-28a expression vetor, the recombinant plasmid was transformed into the *E. coli* host. The soluble recombinant protein was expressed by 0.2 mM of IPTG induction. The results showed that the expression of soluble *pm*MsrA was detected after 2 h of induction, with a molecular mass of approximately 30 kDa. After 14 h of induction, the expression of soluble *pm*MsrA reached the maximal amount (Fig. 2B). Thus, 14 h was chosen as induction time for recombinant *pm*MsrA expression in the following study.

Resting *E. coli* cells expressing recombinant *pm*MsrA were collected and subjected to reaction assay with 2 mM *rac*-**1a** as the model substrate. After 24 h reaction, approximately 51% of *rac*-**1a** was transformed into **1b**. More importantly, the (*S*)-**1a** was totally reduced to **1b** by *pm*MsrA, while the *R*-**1a** was slightly transformed. The *ee* value of (*R*)-**1a** was >99% (Table 1). These results illustrate that the (*R*)-**1a** can be prepared with high enantioselectivity through reductive resolution of *rac*-**1a** by recombinant *pm*MsrA protein.

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