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PAPER

Identification of MsrA Homologues for Preparation of (*R*)-Sulfoxides at High Substrate Concentration

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Here we report a methionine sulfoxide reductase A (MsrA) homologue with extremely high substrate tolerance and wide substrate scope in the biocatalytic preparation of enantiopure sulfoxides. This MsrA homologue which was from *pseudomonas alcaliphila* (named *pa*MsrA) showed good activity and enantioselectivity towards a series of aryl methyl/ ethyl sulfoxides **1a-1k,** with electron-withdrawing or donating substituents at the aromatic ring. Chiral sulfoxides in *R* configration were prepared with approximately 50% of yield and up to 99% enantiomeric excess through asymmetric reductive resolution of racemic sulfoxide catalyzed by the recombinant *pa*MsrA. More importantly, kinetic resolution have been successfully accomplished with high enantioselectivity (*E*>200) at an initial substrate concentration up to 320 mM (approximately 45 g/L), which represents a great improvement in aspect of substrate concentration in biocatalytic preparation of chiral sulfoxides.

Introduction

Chiral sulfoxides are a group of valuable organosulfur chemicals widely used as important intermediates in asymmetric synthesis and medicinal chemistry.[1] Many sulfoxides have high biological activity across a broad range of indications, such as antiulcer agent esomeprazole,[2] potassium channel activator aprikalim,[3] immuno-suppressor oxisurane,[4] etc. Because of the importance of chiral sulfoxides in pharmaceutical industry, as well as their use as chiral auxiliaries in asymmetric synthesis,[5] strategies for their preparation in high optical purity were greatly concerned.[6] As a green approach, biocatalysis has been emerging as an important tool in industrial synthesis of bulk chemicals, pharmaceuticals and agrochemical intermediates.[7] The strategies for biocatalytic preparation of enantiopure sulfoxides can be summarised in two categories, asymmetric oxidation of prochiral sulfides and asymmetric resolution of racemic (*rac*) sulfoxides.[6c, 8] At present, most reported biocatalytic approaches focus on the asymmetric oxidation of sulfides. A lot of enzymes including the BVMO, cytochrome P450 monooxygenase family, peroxidase and toluene dioxygenase have been used to synthesize chiral sulfoxides through whole-cell system or isolated enzyme.[6c, 9]

On the other hand, kinetic resolution of *rac*-sulfoxide has become an effective approach for enantioselective synthesis of chiral sulfoxides, including biological[8a, 10] and chemical[11] ways. Among them, biocatalytic kinetic resolution approach containing two major strategies: the oxidative resolution which asymmetrically oxidize the *rac*-sulfoxides to sulfones, [8a] and the reductive resolution which asymmetrically reduce the *rac*-sulfoxides to the corresponding sulfides.[10] For instance, Xu and colleagues developed a fed-batch reaction of oxidative resolution process using the *Rhodococcus* sp. ECU0066 cells, and the (*S*)-phenyl methyl sulfoxide in 37.8 mM (approximately 5.3 g/L) concentration and 93.7% *ee* was obtained.[8a] Abo and colleagues developed an electrochemical enzymatic deoxygenation system for preparation of chiral sulfoxides by utilizing DMSO reductase.[10a, 10c] Recently, we have established a *pm*MsrA-DTT system which is able to prepare (*R*)-sulfoxides with approximately 45% yield and 94–99% *ee* at substrate concentration up to 200 mM (32 g/L).[10h] This result represents the highest substrate concentration in synthesis of chiral sulfoxides catalyzed by single recombinant enzyme, which is much higher than most of the reported bio-oxidation strategies.[9]

Methionine sulfoxide reductase A (MsrA) is a class of enzyme that specifically reduces the (*S*)-enantiomer of methionine sulfoxide to methionine, which is an important protein repairing enzyme that protects cell from oxidative stress.[12] Thus, it is practicable to develop a high efficient biocatalytic reductive resolution approach for enantioselective synthesis of (*R*)-sulfoxides by MsrA enzyme. Recently, Nosek and co-workers have designed a MsrA-oxaziridine oxidant biphasic reaction system and accomplished up to 90% yield of (*R*)-sulfoxides.[13] Although the theoretical maximum yield of the kinetic resolution is only 50%, the development of enantioselective reduction-oxidation system strongly supports that MsrA could also be used as an excellent biocatalyst for preparing (*R*)-sulfoxides in high yields. Considering the MsrA protein family is widely existed in living species,[12, 14] this study has tested the activities of several

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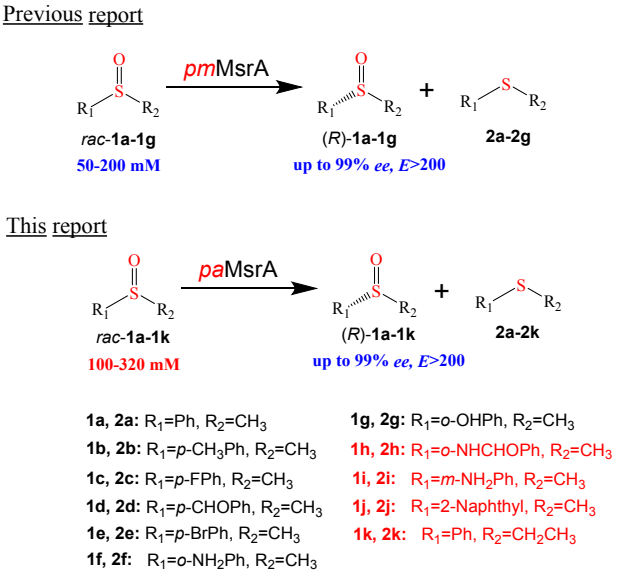
*pm*MsrA homologues aimed to obtain MsrA enzymes with better catalytic performance. Finally, an MsrA homologue from *Pseudomonas alcaliphila* (*pa*MsrA) with wider substrate scope and extremely high substrate tolerance was successfully obtained.



Scheme 1. Enantioselective reduction of *rac*-sulfoxides **1a-1k** to sulfides **2a-2k** by recombinant *pa*MsrA protein, yielding optically pure sulfoxides (*R*)-**1a-1k**.

Results and discussion

Screening and characterization of MsrA homologues

In our previous study, a methionine sulfoxide reductase A gene was cloned from *Pseudomonas monteilii* (*pm*MsrA) and the recombinant protein was used as biocatalyst for preparation of (*R*)-sulfoxides at substrate concentration up to 200 mM.[10h] In this study, we tested the activity of several *pm*MsrA homologues, in order to find MsrA proteins with better catalytic properties for preparing chiral sulfoxides. After sequence alignment using *pm*MsrA as template, four *pm*MsrA homologues with sequence identities from 60%-90% were selected from GenBank database, and named as *pc*MsrA, *pf*MsrA, *pa*MsrA and *vh*MsrA. The detailed protein sequences information was summarized in Table 1. After 14 h of induction by isopropyl-β-D-thiogalactopyranoside (IPTG), all four recombinant proteins were successfully expressed (Figure 1A). The *rac*-**1a** (phenyl methyl sulfoxide, Scheme 1) was used as substrate to test the activity of these enzymes, as well as pH and temperature optima to establish the reaction system. The results showed that all these four recombinant MsrA proteins were active on *rac*-**1a**. Activities of MsrAs from pH 5 to pH 11 in 50 mM phosphate buffer were then analyzed. All four MsrA enzymes showed similar pH profiles and the optimal pH was 8.0 (Figure 1B). These enzymes had more than 70% of activity from pH 7.5 to pH 9.0. Activities from 20 to 60 °C of reaction temperature were also analyzed. These enzymes were most active at 30 °C (Figure 1C). However, the activities of these four enzymes decreased significantly when the reaction temperature reached 40°C, which is quite similar with the *pm*MsrA.[10h] Taken together, pH 8.0 and 30 °C were considered as the optimal reaction conditions.

Table 1. Comparing of *pm*MsrA with 4 homologues.

Name	Organism	Length	Identity [a]
*pm*MsrA	*Pseudomonas monteilii*	222 aa	--
*pc*MsrA	*P. cremoricolorata*	221 aa	90%
*pf*MsrA	*P. fluorescens*	215 aa	81%
*pa*MsrA	*P. alcaliphila*	217 aa	72%
*vh*MsrA	*Vibrio hyugaensis*	212 aa	60%

[a] Sequence identities with *pm*MsrA

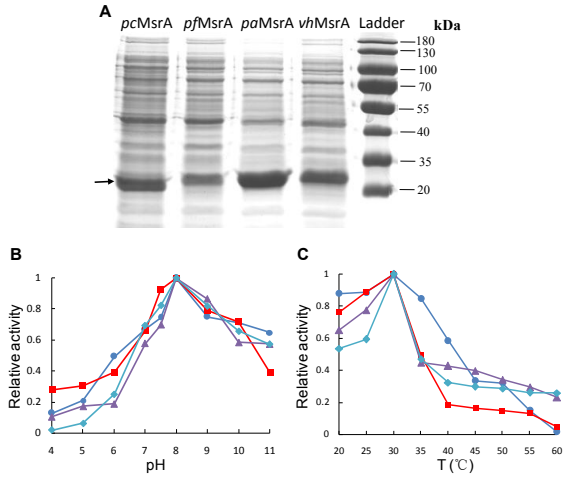


Figure 1. Recombinant expression and characterization of 4 MsrA homologues. A) Gel electrophoresis of 4 recombinant MsrA enzymes, arrow indicates the MsrA protein band. Optimization of B) pH and C) temperature for *pc*MsrA (■), *pf*MsrA (▲), *pa*MsrA (●) and *vh*MsrA (◆) reaction system.

Catalytic analysis of MsrA homologues on kinetic resolution of *rac*-sulfoxides

To test whether these MsrA enzymes could accomplish the kinetic resolution of *rac*-sulfoxides at high substrate concentration, the *rac*-**1a** was used as substrate at the concentration of 100 mM (14 g/L). The crude enzyme-DTT system which we established before was used as the reaction system.[10h] Crude MsrA enzymes (containing approximately 30-55% of MsrA recombinant proteins) were used as biocatalysts at the concentration of 3 g/L of total protein. The results showed that three of the MsrA homologues (*pc*MsrA, *pa*MsrA and *vh*MsrA) exihibited similar activities on *rac*-**1a** compared with *pm*MsrA (Table 2). After 4 h of reaction, (*S*)-**1a** was reduced with high enantioselectivity (*E*>200) and approximately 50% of (*R*)-**1a** with *ee*>90% were obtained by these MsrA homologues. To further compare the activity of *pm*MsrA and these homologues, kinetic resolution of *rac*-**1f** and *rac*-**1g** at the concentration of 100 mM was then performed. In our previous report,[10h] the *pm*MsrA accomlished the kinetic resolution of *rac*-**1f** and *rac*-**1g** at the concentration of 50 mM. When concentration reached 100 mM, the reduction of these two compounds was imcomplete and only less than 30% convension was detected after reaction with *pm*MsrA (Table 2). However, two

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homologues (*pc*MsrA and *pa*MsrA) exihibited fairly good activity towards *rac*-**1f**, which were much better than *pm*MsrA (Table 2). In particular, (*R*)-**1f** with *ee*>99% (*E*>200) was obtained after the reaction by *pa*MsrA. More importantly, *pa*MsrA also accomplished the kinetic reselution of *rac*-**1g**, in which (*R*)-**1g** with *ee*>99% (*E*>200) was obtained after reaction by this enzyme (Table 2). Considering the content of MsrA proteins in crude enzymes were different due to their different expression level, we then purified these recombinant proteins and applied 5 μM of pure MsrA enzymes to further compare their activities. After 1 h of reaction with *rac*-**1g**, 6.8-18% of conversions were detceted (Supporting Information). As expected, the conversion of *rac*-**1g** by *pa*MsrA was obviously higher than other four MsrA homologuses. Taken together, these results suggested that the catalytic properties of *pa*MsrA enzyme was much better than *pm*MsrA and other tested homologuses. Therefore, the *pa*MsrA was selected for our further research on kinetic resolution of *rac*-sulfoxides.

Table 2. Kinetic resolution analysis of *rac*-**1a, 1f, 1g** by *pm*MsrA and 4 homologues. (*t*=4h, substrate concentration=100 mM)

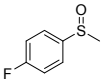
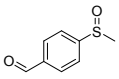
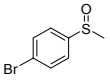
Substrate	Enzyme	Conversion (%) [a]	*ee* (%) [b]	*E* [c]
 **1a**	*pm*MsrA	50.4	>99	>200
	*pc*MsrA	50.3	>99	>200
	*pf*MsrA	37.0	57	119
	*pa*MsrA	50.7	>99	>200
	*vh*MsrA	48.2	90	>200
 **1f**	*pm*MsrA	29.2	n.d.[d]	
	*pc*MsrA	47.4	88	>200
	*pf*MsrA	11.3	n.d.	
	*pa*MsrA	50.2	>99	>200
	*vh*MsrA	21.3	n.d.	
 **1g**	*pm*MsrA	15.9	n.d.	
	*pc*MsrA	30.7	n.d.	
	*pf*MsrA	7.8	n.d.	
	paMsrA	50.1	>99	>200
	*vh*MsrA	13.1	n.d.	

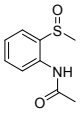
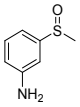
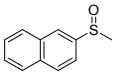
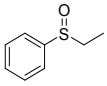
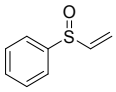
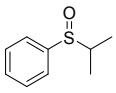
[a] Conversion of **1a, 1f, 1g** to the corresponding products **2a, 2f, 2g** was determined by GC after reaction. [b] The *ee* value of **1g** was determined by GC with CP-ChiraSil-DEX CB column and others were determined by HPLC with AD-H or OD-H chiral column. [c] Calculation of *E* value was as follows: $E=\ln[(1-c)(1-ee)]/\ln[(1-c)(1+ee)]$, where *c* denotes the conversion. [d] "n.d." means not determined due to low conversion.

Investigation of substrate scope of *pa*MsrA

In our previous report,[10h] we found that the *pm*MarA was capable of efficiently preparing a series of (*R*)-sulfoxides **1a-1g**, with electron-withdrawing or donating substituents at the aromatic ring. To test the substrate scope of *pa*MsrA, we also tested these sulfoxides as substrates at a higher concentration (100 mM). Crude *pa*MsrA enzyme was used as biocalyst at the concentration of 3 g/L of total protein (containing approximately 55 μM of *pa*MsrA protein in the reaction mixture). The results showed that all the (*S*)-enationmers were almost completely converted after reaction, and (*R*)-**1a-1g** with *ee*>99% were obtained by this enzyme (Table 3). Then, we test the activity of *pa*MsrA on some other compounds *rac*-**1h-1o**, which were not not working for *pm*MsrA. The results showed that the *pa*MsrA was active on some of these compounds at substrate concentration of 100 mM, eihibiting wider substrate scope compared with our previously reported *pm*MsrA. Among these substrates, the (*S*)-**1h-1j** were reduced with good enantioselectivity and the (*R*)-**1h-1j** with 69-99% *ee* were obtained (Table 3). The high catalytic activity and enatioselectivity of *pa*MsrA on substrates *rac*-**1a-1j** indicates that the substitution on the benzene ring of **1a** affect the enzyme activity slightly, exhibiting an excellent compatibility on this series of substrates. However, the substitution on the methyl group of **1a** affected the activity of *pa*MsrA seriously. Among the substrates *rac*-**1k-1n** (in which the methyl group was replaced by ethyl, chloromethyl, ethenyl and isopropyl groups, respectively), only *rac*-**1k** was active for the enzyme (Table 3). The (*R*)-**1k** with *ee*>99% was abtained with high enantioselectivity at the substrate concentration of 100 mM. The conversion of other three compounds were very low even at the substrate concentration of 10 mM. These results suggest that a methyl or ethyl group was essential for the activity of MsrA enzymes. In addition, we tested the activity of an alkyl sulfoxide *rac*-**1o**. The *pa*MsrA enzyme exhibited high activities on both *R* and *S* configurations of **1o**, in which 91% and 77.8% of conversions were obtained after reaction at the substrate concentrations of 10 mM and 100 mM, respectively. This result demonstrates that the structure of substrate could affect the enantiaoselectivity of MsrA enzyme seriously, while further investigations were needed for explanation of the machenism. Overall, the *pa*MsrA enzyme showed remarkably wide substrate scope for a series of aromatic sulfoxides **1a-1k** at high substrate concentration. Finally, we performed the reactions of **1a-1k** on preparative scale, and 42-51% isolated yields of (*R*)-**1a-1k** were obtained (Table 3).

Table 3. Biocatalytic preparation of (*R*)-**1a-1o** by *pa*MsrA

Substrate	Concentration (mM)	Conversion (%)	*ee* (%) [a]	*E*	Yield (%) [b]
 **1a**	100	50.7	>99	>200	44
 **1b**	100	51.0	>99	>200	43
 **1c**	100	50.7	>99	>200	43
 **1d**	100	50.4	>99	>200	43
 **1e**	100	50.0	>99	>200	44
 **1f**	100	50.2	>99	>200	45

 **1g**	100	50.1	>99	>200	46
 **1h**	100	51.1	95	78	43
 **1i**	100	50.4	>99	>200	44
 **1j**	100	44.4	69	28	51
 **1k**	100	50.9	>99	>200	44
 **1l**	10	7.4	n.d. [c]	--	--
 **1m**	10	11.6	n.d.	--	--
 **1n**	10	33.7	n.d.	--	--
 **1o**	10	91.0	n.d.	--	--
	100	77.8	n.d.	--	--

[a] The *ee* value of **1g-1h** was determined by GC with CP-ChiraSil-DEX CB column and others were determined by HPLC with AD-H or OD-H chiral column. [b] Isolated yield was determined as the ratio of the remaining amount of **1a-1k** after purification to its initial amount. The reaction time for **1c, 1d** and **1o** was 1 h, for **1i** was 0.5 h, and for others was 4 h. [c] "n.d." means not determined.

Investigation of substrate tolerance of *pa*MsrA

Substrate tolerance is a key issue in biocatalytic preparations of chiral sulfoxides for industrial application. In our previous work,[10h] the *pm*MsrA accomplished the kinetic resolution of sulfoxides with high enantioselectivity up to 200 mM of substrate concentration. Our above results about *pa*MsrA implied that this enzyme may have better performance at high substrate concentration. Thus, *rac*-**1a** concentrations from 100-350 mM (14-49 g/L) was used to test the substrate tolerance of *pa*MsrA. The results demonstrated that the reductive resolution of **1a** was successfully accomplished at substrate concentration up to 320 mM (45 g/L) (Figure 2). The (*S*)-**1a** was completely reduced with high enantioselectivity (*E*>200) at this substrate concentration, and approximately 160 mM of (*R*)-**1a** with *ee*> 99% was detected after reaction. We further tested the substrate tolerance of *rac*-**1b-1c** for *pa*MsrA, and the results were quite similar to *rac*-**1a** (Figure 2). The (*R*)-**1b** with 95% *ee* was prepared at 300 mM (43 g/L) of substrate concentration and (*R*)-**1c** with 99% *ee* was prepared at 280 mM (45 g/L) of substrate concentration. To the best of our knowlege, 280-320 mM was the highest susbstrate concentration in biocatalytic preperation of chiral sulfoxides. The substrate tolerance *pa*MsrA was 50 times higher than most of reported native enzymes.[6c] Even compared with our recently reported *pm*MsrA, the substrate concentration of *pa*MsrA was still 1.6-2.0 times higher, representing a significant improvement in the aspect of substrate tolerance. The extremely high substrate tolerance and wide substrate scope of *pa*MsrA implies an excellent industrial prospect. Compared with asymmetric oxidation systems, oxygen is not needed in the MsrA biocatalytic system, which would greatly simplify the process for future industrial application at large scale. Moreover, high cost cofactors like NAD(P)H were not needed in the MsrA reaction system, which also makes it an economical strategy.

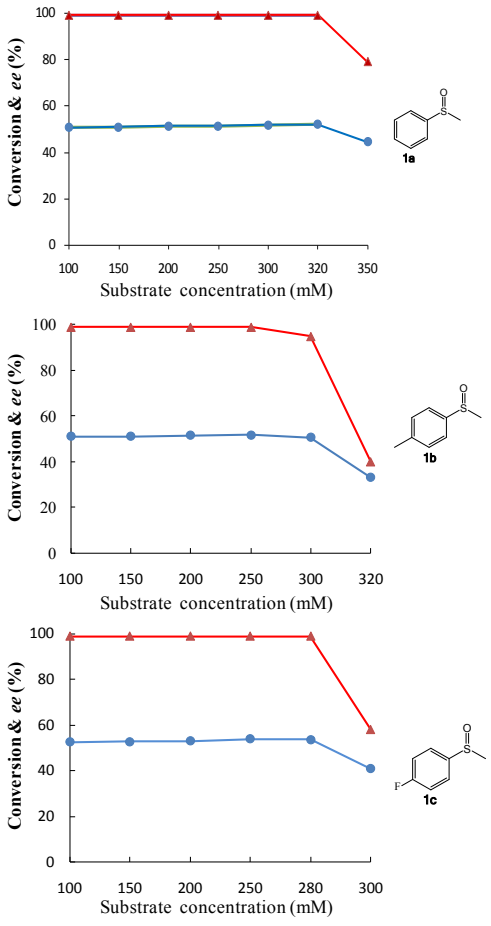


Figure 2. Conversion (●) and *ee* (▲) analysis of *rac*-**1a-1c** after reaction by *pa*MsrA at different substrate concentrations. Crude enzyme concentration: 3 g/L; DTT amount: 0.6-fold of substrate; *t*=4 h.

Conclusions

In summary, four *pm*MsrA homologues were screened from GenBank database and the corresponding recombinant

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proteins were expressed. Three of them showed good activity on *rac*-sulfoxides. More importantly, an MsrA from *P. alcaliphila* species (*pa*MsrA) exhibited remarkably excellent catalytic properties on a series of sulfoxides **1a-1k**. The kinetic resolution of *rac*-sulfoxides was successfully accomplished at up to 320 mM (45 g/L) of substrate concentration catalyzed by *pa*MsrA recombinant enzyme. Our study strongly supports that asymmetric reductive resolution of *rac*-sulfoxides by MsrA would become an effective strategy for green synthesis of optically pure sulfoxides.

Experimental

Screening, cloning and recombinant expression of *pm*MsrA homologues

The protein BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was applied to search MsrA homologues using protein sequence of *pm*MsrA as template. Four MsrA protein sequences with sequence identity from 60%-90% were selected randomly. After obtaining the corresponding nucleotide sequences by tblastn program, the DNA fragments of these MsrA genes were synthesized. The DNA fragment was digested by *Bam*H I and *Hin*d III endonuclease and ligated into the plasmid pET-28a. Afterwards, the recombinant plasmids were transformed into *E. coli* DH5α cells. To induce the soluble expression of recombinant enzymes, the plasmids were transformed into *E. coli* BL21(DE3) cells. An overnight culture of BL21(DE3) cells containing expression plasmid was diluted 1:100 in 1 L LB medium (1% peptone, 0.5% yeast extract, and 1% NaCl) with 50 mg/L of kanamycin and grown at 37 ◦C until it reached an optical density at 600 nm (OD_{600}) of 0.6. Cells were induced with 0.2 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 14 h at 20 ◦C and harvested by centrifugation at 5000 rpm for 10 min. After freezing the cells at -20 ◦C over 1 h, lysis buffer (50 mM phosphate, 1 mg/mL lysozyme, and 5 μg/mL DNase) was added and cells were incubated at room temperature for 1 h. The crude enzyme was obtained by centrifuging for 15 min at 15000 rpm at 4 ◦C. Purification of MsrAs were performed through the His_6-tag in the recombinant proteins. The soluble crude enzymes were mixed with pre-equilibrated Ni-Sepharose resin for 2 h. The flowthrough was removed, and the column was washed with 8 column volumes of wishing buffer (50 mM phosphate, pH 7.4, 150 mM NaCl and 10 mM imidazole). The recombinant proteins were eluted by 2 column volumes of elution buffer (50 mM phosphate, pH 7.4, 150 mM NaCl and 100 mM imidazole). Proteins were quantified by the method of Bradford and subjected to SDS-PAGE gel electrophoresis. The intensities of protein bands was quantified by the software BandScan 5.0 for estimating the content of recombinant MsrA proteins in the crude enzyme.

Relative activity assay of *pm*MsrA

Relative activity of MsrA recombinant enzymes under different pH and temperature conditions was determined by a colorimetric assay based on literatures with modifications.[10h, 15] Specifically, MsrA crude enzyme (0.5 mg/mL), DTT (1 mM), and *rac*-1a (1 mM) were put into 1 mL reaction system containing 50 mM of phosphate buffer. After 20 min reaction under different pH and temperature conditions, the reaction mixture was diluted 20 times by 50 mM phosphate buffer (pH 8.0). Then, diluted samples (100 μL) were transferred into a 96-well plate, and another 100 μL of reaction mixture (4 mM DTNB, 50 mM phosphate buffer, pH 8.0) were added. The plate was incubated under 37 ◦C for 10 min, and a microplate reader was used to detect the absorption at 415 nm (OD_{415}). Proteins extracted from cells containing plasmid pET-28a was used as the control. The decrease of OD_{415} was used to describe the relative MsrA activities.

Biocatalytic kinetic resolution of *rac*-sulfoxides 1a-1o

The substrates *rac*-**1a-1o** were commercially available with analytical grade purity. The process of kinetic resolution was based on our previously reported MsrA-DTT system.[10h] The DTT was added into the reaction buffer (50 mM phosphate, pH 8.0) at the concentration of 0.6-fold of substrate. The crude MsrA enzymes were used at the concentration of 3 g/L of total protein, and the pure enzymes were used at the concentration of 5 μM. For reaction of *rac*-**1e** and **1g**, the substrates were dissolve in 200 μL of methanol and then added into the reaction mixture. For reaction of other substrates, the rac-sulfoxides were directly added into the reaction mixture. Kinetic resolution of *rac*-**1a-1o** was performed at the substrate concentration of 10-320 mM as described in the section of "Results and Discussions". Biocatalytic reactions were performed at 5 mL of reaction volume in a 25 mL glass vial with a screw cap in the incubator at 30 ◦C with shaking at 300 rpm. After incubation for 1-4 h as described in the main text, the reaction mixture was extracted twice by using 10 mL of ethyl acetate with vigorous shaking. The organic layers were collected by centrifugation and then dried by anhydrous sodium sulfate. For separation and purification of sulfoxides after reaction, 5 parallel reactions (5×5 mL, 100 mM of substrates) were performed. After extracting and collecting the reaction mixture, the remaining sulfoxides **1a-1k** were purified by silica gel column chromatography with petroleum ether/ethyl acetate (3:1) as elution solvent.

Analytical methods

Quantification of **1a–1o** and **2a–2o** after reaction were determined by GC with Agilent 7820 GC (Agilent Technologies, Santa Clara, CA) using a HP-5 column (30 m×0.32 mm×0.25 μm) with an FID detector. DMSO was added as an internal standard. The temperature setting was programmed as follows: *T*0: 50 ◦C; d*T*/d*t*: 30 ◦C/min, *T*1: 130 ◦C, 0.5 min; d*T*/d*t*: 20 ◦C/min, *T*2: 150 ◦C, 1.5 min; d*T*/d*t*: 10 ◦C/min, *T*3: 155 ◦C, 1.5 min; d*T*/d*t*: 20 ◦C/min, *T*4: 170 ◦C, 3 min; split ratio 1: 5. The *ee* value of **1g-1h** was determined by GC using a CP-ChiraSil-DEX CB column (25 m×0.25 mm×0.25 mm), and other sulfoxides were determined using a Shimadzu Prominence HPLC on a Daicel™ AD-H or OD-H chiral column (250×4.6 mm, 5 μm) with UV detection at 254 nm, calculated by the equation: *ee*=[([R] -[S])/([R]+[S])] ×100%. Calculation of *E* value for enantioselectivity was as follows: E=ln[(1-c)(1-*ee*)]/ln[(1+c)(1+*ee*)], where c denoted the conversions. Isolated yield of chiral sulfoxides **1a-1k** was determined as the ratio of the remaining sulfoxides after reaction and separation to its initial amount. Optical rotation data of sulfoxides was determined by RUDOLPH research analytical Autopol I-AP. The absolute configuration of **1a-1g**, **1k** was assigned by comparison of the rotation data with previous reports and **1h-1j** was assigned by the above analogies. ^1H 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.

Spectral and *ee* data of 1a-1k after kinetic resolution and purification

Compound (*R*)-**1a**: $[\alpha]_{25}^{D}$=+138.2 (c=2.0, $CHCl_3$) for (*R*), 99% *ee*; lit: $[\alpha]_{25}^{D}$=+152.7 (c=1.0, $CHCl_3$) for (*R*), 99% *ee*;[10h] Daicel OD-H chiral column (250×4.6 mm, 5 μm) at 25℃ with a flow rate of 1 mL/min, and the mobile phase used was 7% isopropyl alcohol/93% n-hexane. Retention time: 15.4 min for (*R*)-**1a** and 20.7 min for (*S*)-**1a**. ^{1}H NMR (400 MHz, CDCl3): δ 2.62 (s, 3H), 7.35-7.44 (m, 3H), 7.53-7.55 (m, 2H); ^{13}C NMR (100 MHz, CDCl3): δ 43.6, 123.3, 129.2, 130.9, 145.3.

Compound (*R*)-**1b**: $[\alpha]_{25}^{D}$=+115.1 (c=2.0, $CHCl_3$) for (*R*), 99% *ee*; lit: $[\alpha]_{25}^{D}$=+128.5 (c=1.0, $CHCl_3$) for (*R*), 99% *ee*.[10h] Daicel OD-H chiral column (250×4.6 mm, 5 μm) at 25℃ with a flow rate of 1 mL/min, and the mobile phase used was 7% isopropyl alcohol/ 93% n-hexane. Retention time: 13.9 min for (*R*)-**1c** and 16.0 min for (*S*)-**1c**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.30-2.32 (m, 3H), 2.58-2.61 (m, 3H),7.21-7.25 (m, 2H),7.42-7.46 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.1, 43.6, 123.3, 129.8, 141.3, 141.9.

Compound (*R*)-**1c**: $[\alpha]_{25}^{D}$=+120.1 (c=2.0, $CHCl_3$) for (*R*), 99% *ee*; lit: $[\alpha]_{25}^{D}$=+131.6 (c=1.0, $CHCl_3$) for (*R*), 99% *ee*.[10h] Daicel OD-H chiral column (250×4.6 mm, 5 μm) at 25℃ with a flow rate of 1 mL/min, and the mobile phase used was 7% isopropyl alcohol/ 93% n-hexane. Retention time: 14.9 min for (*R*)-**1b** and 16.1 min for (*S*)-**1b**. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 2.68 (s, 3H), 7.19 (t, J = 8.5 Hz, 2H), 7.60-7.63 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 44.1, 116.8 (d, *J*= 22.6 Hz, 2C), 125.9 (d, *J*= 8.9 Hz, 1C), 141.0 (d, *J*= 2.9 Hz, 2C), 164.3 (d, *J*=251.5 Hz, 1C).

Compound (*R*)-**1d**: $[\alpha]_{25}^{D}$=+135.01 (c=2.0, $CHCl_3$) for (*R*), 99% *ee*; lit: $[\alpha]_{25}^{D}$=+152.7 (c=1.0, $CHCl_3$) for (*R*), 99% *ee*.[10h] Daicel AD-H chiral column (250×4.6 mm, 5 μm) at 25℃ with a flow rate of 1 mL/min, and the mobile phase used was 10% isopropyl alcohol/ 90% n-hexane. Retention time: 24.1 min for (*R*)-**1d** and 22.7 min for (*S*)-**1d**. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 2.73 (s, 3H), 7.77 (d, *J*=8.2 Hz, 2H), 7.99 (d, *J*=8.2 Hz, 2H), 10.02 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 43.7, 124.2, 130.4, 138.1, 152.3, 191.2.

Compound (*R*)-**1e**: $[\alpha]_{25}^{D}$=+97.3 (c=2.0, $CHCl_3$) for (*R*), 99% *ee*; lit: $[\alpha]_{25}^{D}$=+102.3 (c=1.0, $CHCl_3$) for (*R*), 99% *ee*.[10h] Daicel AD-H chiral column (250×4.6 mm, 5 μm) at 25℃ with a flow rate of 0.8 mL/min, and the mobile phase used was 7% isopropyl alcohol/ 93% n-hexane. Retention time: 32.6 min for (*R*)-**1e** and 31.4 min for (*S*)-**1e**. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 2.65 (s, 3H), 7.46 (d, *J*=8.5 Hz, 2H), 7.59 (d, *J*=8.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 43.9, 125.1, 125.3, 132.5, 144.7.

Compound (*R*)-**1f**: $[\alpha]_{25}^{D}$ =+139.8 (c=2.0, $CHCl_3$) for (*R*), 99% *ee*; lit: $[\alpha]_{25}^{D}$=+144.1 (c=1.0, $CHCl_3$) for (*R*), 98% *ee*.[10h] Daicel OD-H chiral column (250×4.6 mm, 5 μm) at 25℃ with a flow rate of 1 mL/min, and the mobile phase used was 20% isopropyl alcohol/ 80% n-hexane. Retention time: 12.6 min for (*R*)-**1f** and 19.1 min for (*S*)-**1f**. ^{1}H NMR (400 MHz, $CDCl_3$): *δ*2.89 (s, 3H), 4.71 (s, 2H), 6.67-6.74 (m, 2H), 7.19-7.23 (m, 2H);^{13}C NMR (100 MHz, $CDCl_3$): *δ* 38.2, 117.6, 117.7, 123.6, 126.3, 132.5, 147.4.

Compound (*R*)-**1g**: $[\alpha]_{25}^{D}$ =+128.9 (c=2.0, $CHCl_3$) for (*R*), 99% *ee*; lit: $[\alpha]_{25}^{D}$=+133.6 (c=1.0, $CHCl_3$) for (*R*), 94% *ee*.[10h] CP-ChiraSil-DEX CB column (25 m×0.25 mm×0.25 μm) and programmed as follows: *T*0: 50℃; d*T*/d*t*: 30℃/min, *T*1: 130℃,0.5 min; d*T*/d*t*: 20℃/min, *T*2: 150℃, 5 min; d*T*/d*t*: 10℃/min,*T*3: 155℃, 2 min; d*T*/d*t*: 20℃/min, *T*4: 170℃, 2 min; split ratio1: 5. Retention time: 6.397 min for (*R*)-**1g** and 6.604 min for (*S*)-**1g**. ^{1}H NMR (400 MHz, $CDCl_3$):*δ* 2.89 (s, 3H), 6.99 (t, *J* = 7.9 Hz, 2H), 7.32 (t, *J* = 7.6 Hz, 1H), 7.50 (d, *J* = 7.7 Hz, 1H), 10.09 (s, 1H);^{13}C NMR (100 MHz, $CDCl_3$): *δ* 40.9, 117.5, 120.3, 124.5, 126.2, 132.8, 155.9.

Compound (*R*)-**1h**: $[\alpha]_{25}^{D}$ =+117.17 (c=2.0, $CHCl_3$) for (*R*), 95% *ee*; CP-ChiraSil-DEX CB column (25 m×0.25 mm×0.25 μm) and programmed as follows: *T*0: 50℃; d*T*/d*t*: 30℃min^{-1}, *T*1: 130℃, 0.5 min; d*T*/d*t*: 20℃/min, *T*2: 150℃, 5 min; d*T*/d*t*: 10℃min^{-1},*T*3: 155℃, 2 min; d*T*/d*t*: 20℃/min, *T*4: 170℃, 8 min; split ratio1: 5. Retention time: 14.727 min for (*R*)-**1h** and 14.367 min for (*S*)-**1h**. ^{1}H NMR (400 MHz, $CDCl_3$):*δ* 2.13 (s, 3H), 2.83 (s, 3H), 7.06 (t, *J*=7.5 Hz, 1H), 7.24 (d, *J*=6.7 Hz, 1H), 7.39 (d, *J*=7.9 Hz, 1H), 8.29 (d, *J*=8.3 Hz, 1H), 10.40 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 24.8, 40.9, 123.0, 123.8, 125.6, 128.1, 132.5, 139.8, 169.1.

Compound (*R*)-**1i**: $[\alpha]_{25}^{D}$ =+140.55 (c=2.0, $CHCl_3$) for (*R*), 99% *ee*; Daicel OD-H chiral column (250×4.6 mm, 5 μm) at 25℃ with a flow rate of 1 mL/min, and the mobile phase used was 20% isopropyl alcohol/ 80% n-hexane. Retention time: 20.7 min for (*R*)-**1i** and 26.2 min for (*S*)-**1i**. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 2.61 (s, 3H), 4.16 (s, 2H), 6.66-6.68 (m, 1H), 6.67 (d, *J* =7.5 Hz, 1H), 6.94 (s, 1H), 7.15 (t, *J* =7.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 43.5, 108.8, 112.3, 117.2, 129.9, 146.1, 148.0.

Compound (*R*)-**1j**: $[\alpha]_{25}^{D}$ =+172.9 (c=2.0, $CHCl_3$) for (R), 99% *ee*; Daicel OD-H chiral column (250×4.6 mm, 5 μm) at 25℃ with a flow rate of 1 mL/min, and the mobile phase used was 7% isopropyl alcohol/93% n-hexane. Retention time: 16.1 min for (*R*)-**1j** and 22.2 min for (*S*)-**1j**. ^{1}H NMR (400 MHz, CDCl3): *δ* 1.16 (t, *J*=7.4 Hz, 1H), 2.70-2.79 (m, 1H), 2.83-2.92 (m, 1H), 7.46-7.52 (m, 3H), 7.57-7.59 (m, 2H); ^{13}C NMR (100 MHz, CDCl3): *δ* 6.1, 50.3, 124.2, 129.2, 131.0, 143.1.

Compound (*R*)-**1k**: $[\alpha]_{25}^{D}$ =+52.16 (c=2.0, $CHCl_3$) for (R), 69% *ee*; lit: $[\alpha]_{25}^{D}$=+55 (c=0.53, $CHCl_3$) for (*R*), 58% *ee*.[16] Daicel OD-H chiral column (250×4.6 mm, 5 μm) at 25℃ with aflow rate of 1 mL/min, and the mobile phase used was 20% isopropyl alcohol/80% n-hexane. Retention time: 14.5 min for (*R*)-**1k** and 16.7 min for (*S*)-**1k**. ^{1}H NMR (400 MHz, CDCl3): *δ* 2.75 (s, 3H), 7.53-7.57 (m, 3H), 7.84-7.94 (m, 2H), 7.93 (d, *J*= 8.6 Hz, 1H), 8.17 (s, 1H); ^{13}C NMR (100 MHz, CDCl3): *δ* 119.4, 124.0, 127.3, 127.8, 128.0, 128.4, 129.6, 132.8, 134.3, 142.6.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 (a) B. M. Trost, M. Rao, Angew. Chem. Int. Ed. 2015, **54**, 5026-5043; (b) S. Otocka, M. Kwiatkowska, L. Madalinska, P. Kiełbasinski, Chem. Rev.2017, **117**, 4147-4181; (c) J. Han, V. A. Soloshonok, K. D. Klika, J. Drabowicz, A. Wzorek, Chem. Soc. Rev. 2017, **47**, 1307-1350.

2 H. J. Federsel, Chirality 2003, **15**, S128-S142.

3 K. Okamoto, T. J. Nishino, Biol. Chem. 1995, **270**, 7816-7821.

4 N. Farrell; D. M. Kiley, W. Schmidt, M. P. Hacker, Inorg. Chem. 1990, **29**, 397−403.

5 (a) E. Wojaczynska, J. Wojaczynski, Chem. Rev. 2010, **110**, 4303-4356; (b) H. Pellissier, Tetrahedron, 2006, **37**, 5559-5601.

6 (a) I. Fernández, N. Khiar, Chem. Rev. 2003, **103**, 3651-3705; (b) J. Wang, M. Frings, C. Bolm, Chem. Eur. J. 2014, **20**, 966-969; (c) T. Matsui, Y. Dekishima, M. Ueda, Appl. Microbiol. Biotechnol. 2014, **98**, 7699-7706.

7 (a) S. Claudia, L. Fernando, Microbial Biotech. 2016, **9**, 601-609; (b) J. Wachtmeister, D. Rother, Current Opinion in Biotechnology, 2016, **42**, 169-177; (c) R. A. Sheldon, P. C. Pereira, Chem. Soc. Rev. 2017, **46**, 2678-2691; (d) R. A. Sheldon, J. M. Woodley, Chem Rev. 2018, **118**, 801-838.

8 (a) H. R. Luckarift, H. Dalton, N. D. Sharma, D. R. Boyd, R. A. Holt, Appl. Microbiol. Biotechnol. 2004, **65**, 678-685; (b) A. T. Li, H. L. Yu, J. D. Zhang, J. H. Xu, G. Q. Lin, Bioresour. Technol. 2011, **102**, 1537-1542; (c) J. Han, V. A. Soloshonok, K. D. Klika, J. Drabowicz, A. Wzorek, Chem. Soc. Rev. 2018, **47**, 1307-1350.

9 (a) G. Bertoni, F. Bolognese, E. Galli, P. Barbieri, Appl. Environ. Microbiol. 1996, **62**, 3704-3711; (b) G. D. Gonzalo, D. E. T. Pazmino, G. Ottolina, M. W. Fraaije, G. Carrea, Tetrahedron: Asymmetry, 2006, **17**, 130-135; (c) J. D. Zhang, A. T. Li, J. H. Xu, Bioprocess Biosyst. Eng. 2010, **33**, 1043-1049. (d) X. H. Zhai, Y. H. Ma, D. Y. Lai,, S. Zhou, Z. M. Chen, J. Ind. Microbiol. Biotechnol. 2013, **40**, 797-803; (e) Y. Chen, J. Zhuo, D. Zheng, S. Tian, Z. Li, J. Mol. Catal. B, 2014, **106**, 100-104; (f) T. I. Kylosova, A. A. Elkina, V. V. Grishko, I. B. Ivshina, Journal of Molecular Catalysis B: Enzymatic, 2016, **123**, 8-13. (e) W. R. F. Goundry, B. Adams, H. Benson, J. Demeritt, S. Mckown, K. Mulholland, A. Robertson, P. Siedlecki, P. Tomlin, K. Vare, Org. Process Res. Dev. 2017, **21**: 107-113.

10 (a) M. Abo, M. Tachibana, A. Okubo, S. Yarnazaki, Bioorg Med Chem 1995, **3**, 109-112; (b)S. P. Hanlon, D. L. Graham, P. J. Hogan, R. A. Holt, C. D. Reeve, A. L. Shaw, A. G. McEwan, Microbiology 1998, **144**, 2247-2253; (c) M. Abo, M. Dejima, F. Asano, A. Okubo, S. Yarnazaki, Tetrahedron: Asymmetry 2000, **3**, 823-828; (d) D. R. Boyd, N. D. Sharma, A. W. T. King, S. D. Shepherd, C. C. R. Allen, R. A. Holt, H. R. Luckarift, H. Dalton, Org. Biomol. Chem. 2004, **2**, 554-561; (e) M. Tudorachea, S. Nicab, E. Barthab, I. Lupanc, V. I. Parvulescua, Applied Catalysis A: General, 2012, **441-442**, 42-46; (f) J. Yang, Z. Yuan, Z, Yang, J. Zhao, M. Yang, X. Cheng, G. Ou, Y. Chen, Journal of Molecular Catalysis B: Enzymatic 2016, **133**, S588–S592; (g) C. Achilli, A. Ciana, G. Minetti, Tetrahedron Lett. 2017, **58**, 4781–4782; (h) L. Peng, Y. Wen, Y. Chen, Z. Yuan, Y. Zhou, X. Cheng,Y.Chen, J. Yang, ChemCatChem 2018, **10**, 3284-3290.

11 (a) P. K. Dornan, K. G. M. Kou, K. N. Houk, V. M. Dong, J. Am. Chem. Soc. 2014, **136**, 291-298. (b) J. R. Lao, H. Fernández-Pérez, A. Vidal-Ferran, Org. Lett. 2015, **17**, 4114-4117.

12 (a) M. Kantorow, J. R. Hawse, T. L. Cowell, S. Benhamed, G. O. Pizarro, V. N.Reddy, J. F. Hejtmancik, Proc. Natl. Acad. Sci. USA 2004, **101**, 9654-9659; (b) S. Boschi-Muller, G. Branlant, Bioorg Chem. 2014, **57**, 222-230; (c) C. Yin, L. Zheng, J. Zhu, L. Chen, A. Ma, Appl Microbiol Biotechnol. 2015, **99**, 3115-3126.

13 V. Nosek, J. Míšek, Angew Chem Int Ed Engl. 2018, **57**, 9849-9852.

14 (a) H. Weissbach, L. Resnick, N. Brot, Biochim Biophys Acta. 2005, **1703**, 203-212; (b) N. Rouhier, C. Vieira Dos Santos, L. Tarrago, P. Rey, Photosynth Res. 2006, **89**, 247-262; (c) C. Dai, M. H. Wang, Mol Biol Rep. 2012, **39**, 6297-6308.

15 P. F. Wu, Z. Zhang, X. L. Guan, Y. L. Li, J. H. Zeng, J. J. Zhang, L. H. Long, Z. L. Hu, F. Wang, J. G. Chen, Enzyme Microb. Technol. 2013, **53**, 391-397.

16 F. Gelat, J. Jayashankaran, J. F. Lohier, A. C. Gaumont, S. Perrio, Organic letters 2011, **13**, 3170-3173.

An MsrA homologue with extremely high substrate tolerance and wide substrate scope for the biocatalytic preparation of enantiopure sulfoxides

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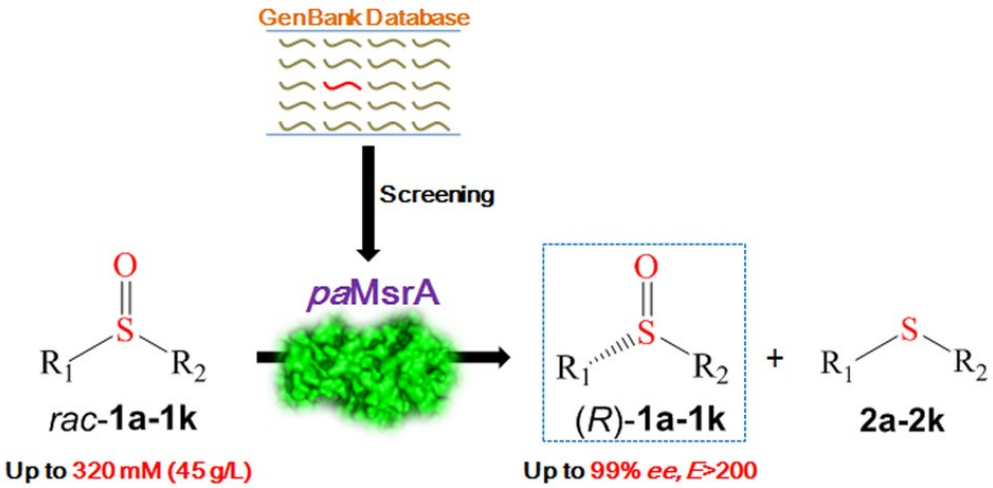

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