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Enzymatic Kinetic Resolution of Chiral Sulfoxides - An Enantiocomplementary Approach

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A new enzymatic assay for the preparation of chiral sulfoxides that is enantiocomplementary to the known (*S*)-enatiomer-reducing activity of methionine sulfoxide reductase A (MsrA) is described. To this end, we have utilized the enzyme DMSO reductase (DmsABC), recentlly discovered by us being highly upregulated in the stationary phase *E. coli* bacteria.

Biocatalysis has become one of the essential modalities in modern asymmetric catalysis along with the transition metal catalysis and organocatalysis.[1–5] The use of enzymes for preparation of enantioenriched chiral molecules can be highly efficient in terms of activity and enantioselectivity. Nevertheless, the common drawback of natural enzymes is their high substrate specificity and the lack of natural enantiocomplementary counterparts. These issues can now be addressed by methods of directed evolution of enzymes that were pioneered by Arnold in the nineties and has since become an indispensable tool for generation of tailor-made enzymes for production of high-value chemicals in both academia and industry.[6–14] It has been shown that natural enantioselective enzymes can be laboratory-evolved to exhibit opposite enantioselectivity.[15,16] However, such a process is relatively tedious, and the resulting mutants do not necessarily have the same level of activity and selectivity as the wild type.

We have previously reported a general chemoenzymatic method for deracemisation of chiral sulfoxides.[17] This method utilizes methionine sulfoxide redutase A (MsrA), a natural enzyme capable of highly efficient kinetic resolution of racemic sulfoxides (Scheme 1). MsrA reacts exclusively with (*S*)-enantiomers of an exceptionally wide range of racemic sulfoxides and thus provids enantiomerically pure (*R*)-sulfoxides. There exists a natural enantiocomplementary enzyme methionine reductase B (MsrB) that reduces the (*R*)-sulfoxide.[18,19] We envisaged that the existence of this MsrB would allow us to develop an enantiocomplementary enzymatic process for asymmetric preparation of (*S*)-sulfoxides. However, the low activity and high substrate specificity of natural MsrBs we tested, prevented us from the development of a practical and general method for kinetic resolution of sulfoxides. This phenomenon of lower activity and high substrate specificity of natural MsrBs as compared to MsrAs was observed previously and seems to be general.[18] During our research into the stereochemical aspects of oxidative stress in *E. coli* we developed chiral fluorescent probes (*S*)- and (*R*)-Sulfox-1.[20] By employing these probes, we have discovered a new (*R*)-sulfoxide-reducing activity in *E. coli* under stress conditions. The underlying cause of the activity was unambiguously assigned to DMSO reductase (DmsABC).[21] *E. coli* DMSO reductase is a known enzyme but its role in protection against oxidative stress has not been reported before.[22,23] As far as its stereoselectivity of reduction is concerned, there were reports on enantioselective reduction of sulfoxides in whole *E. coli* cells, where DMSO reductase was suspected for the activity, however no direct evidence was provided.[24–26] The observed very high activity and enantioselectivity of DMSO reductase with our probes under specific condition prompted us to test this enzyme for enantiocomplementary kinetic resolution of racemic sulfoxides.

Herein, we report on the new enzymatic protocol for efficient and general kinetic resolution of chiral sulfoxides that is enantiocomplementary to the known method using MsrA enzyme.

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† Footnotes relating to the title and/or authors should appear here.
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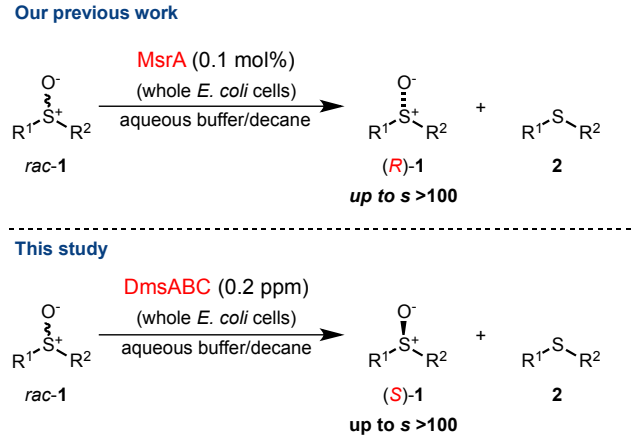
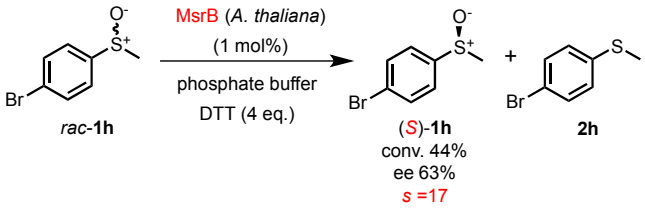


Scheme 1 Methods for enzymatic kinetic resolution of chiral sulfoxides.

Our quest for an efficient enantiocomplementary method for kinetic resolution of sulfoxides commenced with the recombinant expression of MsrBs with highest reported activities (see the ESI).[27–29] Among those, MsrB from *A. thaliana* turned out to be the most active and enantioselective (Scheme 2). However, as compared with MsrA, the model reaction employing sulfoxide **1h** required ten-fold excess of the enzyme MsrB (1 mol%) compared to MsrA (0.1 mol%) in order to reach a reasonable conversion (44%) in 44 hours. The enantiomeric excess of the isolated product was 63% giving the selectivity factor of 17 (calculated according to the formula s=ln[(1-c)(1-ee)]/ln[(1-c)(1+ee)] where c is conversion and ee is enantiomeric excess).[30] This experiment indicated rather poor performance of the enzyme in the kinetic resolution of sulfoxides. At this point, we concluded that the known MsrBs are unlikely to be the ideal choice for development of a general and efficient enantiocomplementary method.

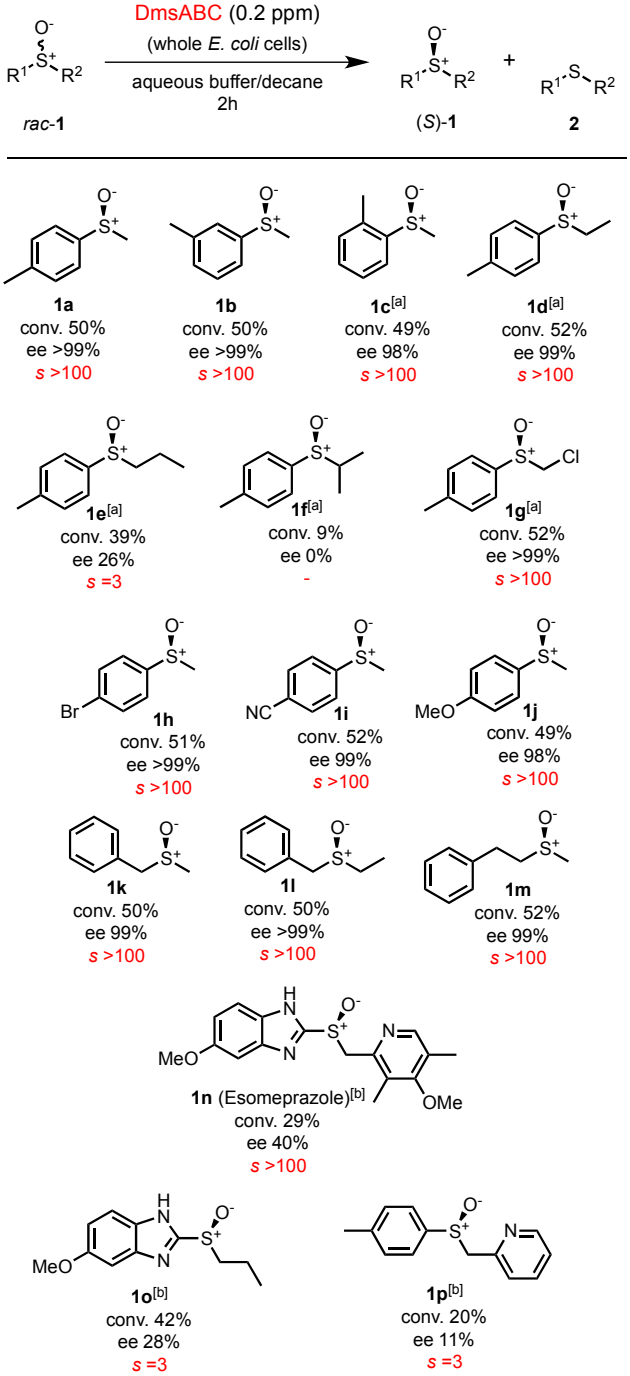


Scheme 2 Kinetic resolution of a model substrate **1h** with methionine sulfoxide reductase B from *A. thaliana*.

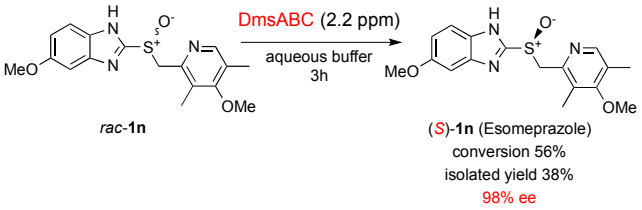
Fortunately, other lines of our research provided us with the discovery that DmsABC enzyme that is highly upregulated under stationary phase growth in *E. coli* possesses the desired enantiocomplementary activity. DmsABC is a membrane-associated enzyme consisting of three subunits that are required for the activity.[22] However all our attempts at isolation of the DmsABC reductase from a membrane fraction led to significant decrease in activity. Therefore, we resolved to use the whole *E. coli* cells format of the reaction. It should be noted that also the *E. coli* crude membrane fraction can be utilized for the kinetic resolution of sulfoxides when supplemented with NADH as an external reductant. However, the activity of DmsABC reductase turned out to be strongly dependent on the process of preparation of the membrane fraction, which led to a rather low reproducibility. Another advantage of the whole cell format is that no external reductant is required as the intracellular NADH drives the reduction. We used stationary phase growth *E. coli* population for the reaction as the activity of DmsABC reductase is naturally upregulated under this condition.[21] It has been shown that natural activity of enantiocomplementary MsrA is also upregulated at the stationary phase.[31] Our previous measurement with Sulfox-1 probes indicated that stationary phase *E. coli* activity of MsrA is singnificantly lower than that of DmsABC reductase but still measurable. Thus, we used MsrA knock-out strain for kinetic resolution experiments in order to prevent this undesired activity. The preliminary experiment was performed with **1a** as a model substrate. *E. coli* cells (MsrA knock out – KEIO collection ID JW4178)[32] were harvested at the stationary phase (OD_{600}=3), resuspended in M9 minimal buffer (OD_{600}=70; 2 ml) and substrate **1a** (65 µmol; 8 ml M9 minimal buffer) and decane (5 vol %) were added and the resulting mixture was incubated at 37 °C. Conversion and enantiomeric excess of the sulfoxide **1a** were monitored at various timepoints by HPLC. Delightfully, after two hours the observed conversion was 51% and ee >99% for the (*S*)-enantiomer giving the selectivity factor s >100. In order to assess the amount of DmsABC reductase in the reaction mixture, proteomic analysis of the harvested cells was performed. The relative intensity of all proteins was related to the total protein concentration. Based on this analysis the amount of DmsABC reductase in the reaction mixture was estimated to be 0.00002 mol% or 0.2 ppm. This level of activity is in agreement with the previously reported data for the purified enzyme and documents the exceptional catalytic performance enzymes may possess.[22]

Next, we assessed the substrate scope of the reaction. A wide range of aryl alkyl and alkyl alkyl sulfoxides **1a**-**1m** were successfully resolved with excellent enantioselectivity (s factor >100)(Scheme 3). Similarly to MsrA, DmsABC reductase can efficiently resolved various aryl melthyl/ethyl sulfoxides. Also, chiral sulfoxides bearing two adjacent sp^3 carbons, which are often difficult substrates for chemical catalysis can be resolved with high enantioselectivity. Propyl tolyl sulfoxide **1e** that is unreactive with MsrA can react with DmsABC reductase but the enantioselectivity is very low (s=3). Isomeric isopropyl tolyl sulfoxide **1f** is even less reactive suggesting the sensitivity of the enzyme to the branching at the alpha carbon of the sulfoxide moiety. Interestingly, chloromethyl tolyl sulfoxide **1g** that is also unreactive with MsrA proved to be a good substrate for DmsABC reductase affording the product with high enantioselectivity (s>100). This chloromethyl substitution enables further synthetic modifications by means of nucleophilic substitution or reactions with organometallic species and thus further expands the scope of accessible chiral sulfoxides in an enantiomerically pure form.[33–36] As the enzyme provides (*S*)-enatiomers of sulfoxides, we also tested Omeprazole as a substrate in order to obtain the blockbuster drug Esomeprazole (**1n**). Gratifyingly, racemic Omeprazole was successfully resolved with an excellent selectivity factor s >100. This rather surprising reactivity

prompted us to test also substrates **1o** and **1p** in order to identify the substitution pattern required for high enantioselectivity compared with substrate **1e**. These experiments showed that neither of these substrates is resolved with high enantioselectivity and thus other steric/electronic effects must be considered to explain the high enantioselectivity for substrate **1n**. In order to further demonstrate the utility of the enzymatic kinetic resolution, we resolved Omeprazole into blockbuster drug Esomeprazole on a larger scale. Due to the low solubility of Omeprazole in the reaction buffer, pH was raised to 9. Indeed, this modification of the protocol increased the solubility of Omeprazole and also suppressed the generation of undesired side products. Furthermore, decane co-solvent was not required in this case. 50 mg of omeprazole was resolved to obtain Esomeprazole (**1n**) in 38% isolated yield (conversion 56%) with the enantiomeric excess of 98%. This proof-of-principle experiment was performed in only 100 ml reaction volume without any extensive optimisation. For the preparation of larger quantities of Esomeprazole, an increased reaction volume and/or further optimisation of the reaction conditions (e.g. flow reactor) would be required.



Scheme 3 Substrate scope of the DmsABC catalyzed kinetic resolution of chiral sulfoxides. [a] 0.4 ppm of DmsABC. [b] 2.0 ppm of DmsABC. For details, see the ESI.



Scheme 4 Asymmetric preparation of Esomeprazole by means of DmsABC catalysed kinetic resolution.

In conclusion, we have developed a new enantiocomplementary enzymatic protocol for highly efficient kinetic resolution of chiral sulfoxides. Remarkably, a wide range of substrates can be resolved with an excellent enantioselectivity, which is a feature that is exceptional even among other enantioselective enzymes. Although, the principal enzyme DmsABC reductase is a multidomain, multi cofactor enzyme complex that is not easily isolated, we came up with a simple whole-cell format of the reaction that can be easily utilized for an efficient resolution providing a blockbuster drug Esomeprazole. Remarkably, the catalyst loading of 0.2 ppm demonstrates the efficiency that can be achieved with biocatalysis. In a broader sense, we show that new useful enzymatic activities can be discovered even in the well-studied organisms such as *E. coli*. Thus, we believe, that natural sources still represent an untapped potential for the discovery of new reactivities.

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Conflicts of interest

There are no conflicts of interest.

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