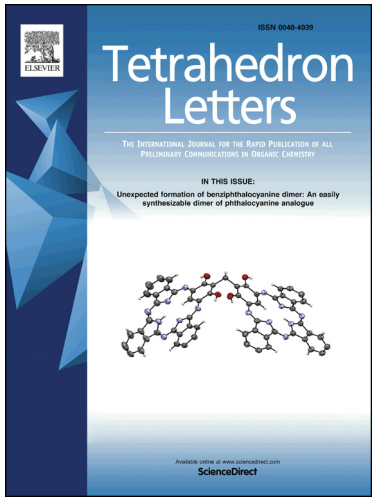


Kinetic resolution of phenyl methyl sulfoxides by mammalian methionine sulfoxide reductase A

Cesare Achilli, Annarita Ciana, Giampaolo Minetti

PII: S0040-4039(17)31425-9
DOI: https://doi.org/10.1016/j.tetlet.2017.11.022
Reference: TETL 49465

To appear in: *Tetrahedron Letters*

Received Date: 27 September 2017
Revised Date: 6 November 2017
Accepted Date: 10 November 2017

Please cite this article as: Achilli, C., Ciana, A., Minetti, G., Kinetic resolution of phenyl methyl sulfoxides by mammalian methionine sulfoxide reductase A, *Tetrahedron Letters* (2017), doi: https://doi.org/10.1016/j.tetlet.2017.11.022

Tetrahedron Letters
journal homepage: www.elsevier.com

Kinetic resolution of phenyl methyl sulfoxides by mammalian methionine sulfoxide reductase A

Cesare Achilli[a] , Annarita Ciana[a] , Giampaolo Minetti[a,*]

[a] *University of Pavia, Department of Biology and Biotechnology, Laboratories of Biochemistry, via Bassi 21, 27100 Pavia, Italy*

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Aryl methyl sulfoxide
Kinetic resolution
Chiral auxiliary
Methionine sulfoxide reductase
Enzyme

ABSTRACT

Chiral sulfoxides are widely used in organic synthesis as chiral auxiliaries. There are numerous strategies for the preparation of enantiomerically pure sulfoxides, based either on the enantioselective oxidation of sulphides or the enantiospecific reduction of sulfoxides. For both cases, bioconversion techniques have been developed and proposed for large-scale synthesis. Methionine sulfoxide reductase enzymes (MsrA and MsrB) catalyse the stereoselective conversion of methionine sulfoxide to methionine. MsrA can also catalyse the reduction of other exogenous sulfoxides, including *p*-tolyl methyl sulfoxide. However, the stereoselectivity towards this type of substrate is not yet well characterized. The activity and enantioselectivity of MsrA toward several aryl methyl sulfoxides is presented in this paper.

In the last two decades, enantiomerically pure chiral sulfoxides have found many applications in organic synthesis, mainly because they can act as excellent chiral auxiliaries, capable of guiding carbon-to-carbon bond formation in an enantioselective manner, but also in the reduction of carbonyl compounds and olefins.[1] There are three factors behind the success of these molecules as chiral auxiliaries: their high optical stability, their efficiency in transferring chiral information, and the availability of both enantiomers in optically pure form. The main procedures for the large-scale preparation of optically pure sulfoxides by oxidation of the respective sulphide are based on the modified Sharpless method or on the use of chiral oxaziridines. In recent years, bioconversion techniques based on oxidative enzymes such as monooxygenase and chloroperoxidase have been increasingly developed.[2]

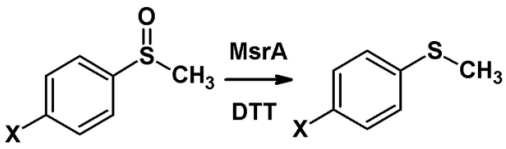
An alternative to obtaining optically pure sulfoxides is the kinetic resolution of a racemic mixture, which can be achieved by means of an enantiospecific reaction involving the transformation of only one of the enantiomers. For this purpose the alternatives are oxidation to the sulfone or reduction to the sulphide.[3] More recently, the ability of the bacterial enzyme dimethyl sulfoxide reductase to catalyze the reduction of a wide range of aryl alkyl sulfoxides has been discovered, in some cases showing high enantiospecificity.[4] Another class of enzymes that has biologically evolved the ability to reduce the sulfoxide group is methionine sulfoxide reductase (Msr).[5] The Msrs differ for their stereospecificity towards the two diastereomeric forms of methionine sulfoxide that may result from aberrant oxidation of methionine (as the free amino acid or inserted into proteins) during cellular metabolism. MsrAs reduce (S_C)methionine-(S_S)sulfoxide, whereas MsrBs reduce (S_C)methionine-(R_S)sulfoxide. The physiological electron donor for Msrs is the thioredoxin system which, *in vitro*, can be replaced by 1,4-dithiothreitol (DTT) or 1,4-dithioerythritol (DTE).[6] Moskovitz and co-workers have shown that mammalian MsrA has, *in vitro*, high enzymatic activity against (S_S)*p*-tolyl methyl sulfoxide, while the substitution of methyl with the phenyl group completely suppresses the catalytic activity, probably due to the excessive steric hindrance of the substrate.[7] However, the activity towards the R_S enantiomer has not been tested, and it is not possible assume *a priori* that an enzyme also maintains its natural stereospecificity towards synthetic substrates. While the present article was under review, data were reported on the asymmetric resolution of a series of chloro-substituted phenyl methyl sulfoxides by recombinant MsrA from *Pseudomonas montelii* overexpressed in *Escherichia coli* and used in a whole cell system. An enantioselectivity towards the *S* enantiomer was observed, with enantiomeric excesses of 93.2% (*ortho*), 61.2% (*meta*) and 97.0% (*para*).[8]

Herein, we have evaluated the enantiospecificity of mammalian MsrA towards *p*-tolyl methyl sulfoxide. In addition, the performance in the enantiospecific reduction of a series of phenyl methyl sulfoxides has also been tested. Mammalian MsrA was obtained by recombinant DNA technology, the substrates were in the racemic form and DTT was used as an electron donor. Direct reduction of the sulfoxide to sulphide by DTT alone was found to be negligible under our experimental conditions. The degree of conversion was determined by reverse phase HPLC analysis and the enantioselectivity was checked by HPLC analysis on a column packed with cellulose tribenzoate-

* Corresponding author. Tel.: +39-0382-987891; fax: +39-0382-987240; e-mail: minetti@unipv.it

coated silica, which is able to resolve the two enantiomers of these sulfoxides, as previously demonstrated[9] (experimental details are provided in the ESI). Mammalian MsrA displayed an elevated enantiospecificity for the (S_S)*p*-tolyl methyl sulfoxide, confirming what is already known with respect to the natural substrate (Table 1). The same level of activity and the same enantiospecificity were also observed for the other substrates that were tested (Table 1). The behavior towards *p*-chlorophenyl methyl sulfoxide was consistent with that recently observed for MsrA from *Pseudomonas monteilii*.[8] The stereospecificity of mammalian MsrA did not seem to depend on the identity of the substituents in the *para* position of the aromatic ring. It was also observed that mammalian MsrA exhibited a negligible activity against phenyl ethyl sulfoxide according to what was already reported in the literature, where substituting methyl with larger moieties, such as phenyl, caused a decrease in the enzymatic activity of mammalian MsrA, whereas the other substituent adjacent to the reaction center had only a minor effect on the enzyme activity.[7]

Table 1. Values of enantiomeric excess of R_S and degree of conversion obtained after the reduction of aryl methyl sulfoxides by mammalian MsrA



Substrate	Enantiomeric excess of R_S (%)	Degree of conversion (%)[b]
phenyl methyl sulfoxide	90	46
p-tolyl methyl sulfoxide	96	44
p-fluorophenyl methyl sulfoxide[a]	85	42
p-chlorophenyl methyl sulfoxide[a]	91	41
p-bromophenyl methyl sulfoxide[a]	92	51

[a] These sulfoxides were not commercially available, therefore they were prepared by oxidation of the respective sulphides with peroxydisulphate, and purified by recrystallization as previously described.[10]

[b] Degree of conversion is expressed as the ratio between the moles of sulphide and the moles of the initial sulfoxide.

In light of the data reported herein, mammalian MsrA represents a new enzymatic method for the kinetic resolution of racemic mixtures of aryl methyl sulfoxide, yielding the R_S enantiomer with a good degree of optical purity. Mammalian MsrA, thanks to recombinant DNA technology, can be expressed in prokaryotic cells at very high levels and could find application in large-scale strategies of bioconversion using raw cell extracts or directly into intact cultured cells. The preference for substrates having a methyl group adjacent to the sulfoxide moiety could also be exploited to reduce complex molecules containing more sulfoxide units, since mammalian MsrA would be able to select the reaction site based on the different steric hindrance. The behavior of other MsrA isoforms remains to be explored, as it is the ability of members of the MsrB family (with the noteworthy presence of a selenoenzyme[11]) to reduce unnatural sulfoxides, and whether they are specific towards the R_S enantiomer.

Acknowledgments

This work was supported with FAR funds from the University of Pavia to GM. Authors would like to thank Prof. Herbert Weissbach for having kindly provided the recombinant bacteria for the overexpression and purification of MsrA.

The authors declare that they have no conflict of interest.

References and notes

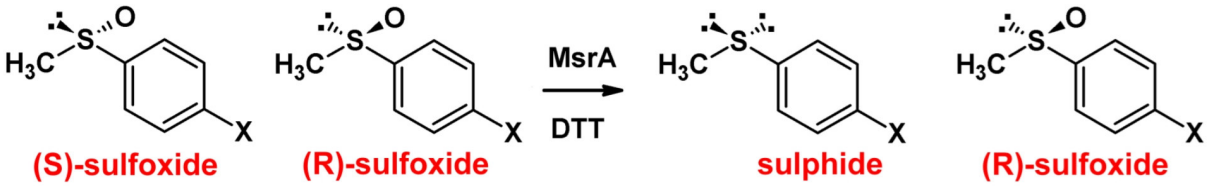
1. Pellissier, H. *Tetrahedron* **2006**, *62*, 5559-5601
2. Fernàndez, I.; Khiar, N. *Chem. Rev.* **2003**, *103,* 3651-3705
3. O'Mahony, G. E.; Kelly, P.; Lawrence, S. E.; Maguire, A. R. *Arkivoc.* **2011**, *2011*, 1-110
4. Hanlon, S. P.; Graham, D. L.; Hogan, P. J.; Holt, R. A.; Reeve, C. D.; Shaw, A. L.; McEwan, A. G. *Microbiology* **1998**, *144*, 2247-2253
5. Achilli, C.; Ciana, A.; Minetti, G. *Biofactors* **2015**, *41*, 135-152
6. Minetti, G.; Balduini, C.; Brovelli, *A. Ital. J. Biochem.* **1994**, *43*, 273-283
7. Moskovitz, J.; Weissbach, H.; Brot, N. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 2095-2099
8. Yang J.; Yuan Z.; Zhou Y.; Zhao J.; Yang M.; Cheng X.; Ou G.; Chen Y. *J. Mol. Catal. B Enzym.* **2017** In Press
9. Abo, M.; Okubo, A.; Yamazaki, S. *Tetrahedron-Asymmetr.* **1997**, *8*, 345-348
10. Arumugan, A.; Srinivasan, C.; Kuthalingam, P. *Ind. J. Chem.* **1978** *16*, 478-480
11. Achilli,C.; Ciana, A.; Rossi, A.; Balduini, C.; Minetti, G. *J. Leukoc. Biol.* **2008** 83, 181-189

Graphical Abstract

Kinetic resolution of phenyl methyl sulfoxides by mammalian methionine sulfoxide reductase A

Cesare Achilli, Annarita Ciana, Giampaolo Minetti

Leave this area blank for abstract info.



Chiral sulfoxides are of great interest as chiral auxiliaries in organic synthesis

An enzymatic kinetic resolution of aryl methyl sulfoxides is proposed

Methionine sulfoxide reductase A (MsrA) reduces in vivo S_c-Met-S_s-sulfoxide to S_c-Met

We show here that MsrA reduces the S enantiomer of some aryl methyl sulfoxides

MsrA is suggested for the large-scale preparation of optically pure sulfoxides