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Unusual stereoselectivity of methionine-γ-lyase from *Citrobacterfreundii* toward diastereomeric (*S*)-methionine *S*-oxide

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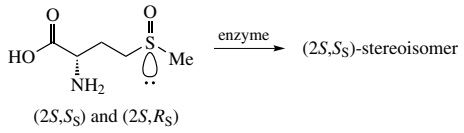
Using a diastereomeric mixture of (*S*)-methionine *S*-oxide as an example, kinetic preference of methionine-γ-lyase toward a stereogenic center at the γ-sulfur atom of the (2*S*,R_S) diastereomer was discovered for the first time.



(2*S*,S_S) and (2*S*,R_S)

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The biocatalytic cleavage of racemates has been intensely studied in recent years.[1,2] Lipases are widely used in organic synthesis to separate racemic amines and diamines,[3,4] alcohols,[5] diols[6] and hydroxy esters,[7] and to perform stereoselective reactions, *e.g.*, the Michael reaction.[8] For example, previously we used PPL lipase for hydrolytic cleavage of esters of a number of amino acids[9,10] and for enantioselective acylation of venfalaxine with vinyl acetate,[11] and utilized methionine-γ-lyase for the cleavage of phosphorus analogues of sulfur-substituted amino acids.[12,13]

The successful stereoselective cleavage of unusual substrates comprising a few chiral centers by enzymes is much less common. As a rule, the known examples include the preliminary separation of racemic diastereomers by standard methods, most commonly by chromatography, followed by enzymatic cleavage of the substrates into individual enantiomers.[6,14–16] In this case, the cleavage of both diastereomers usually occurs with the same result and with similar stereospecificity.

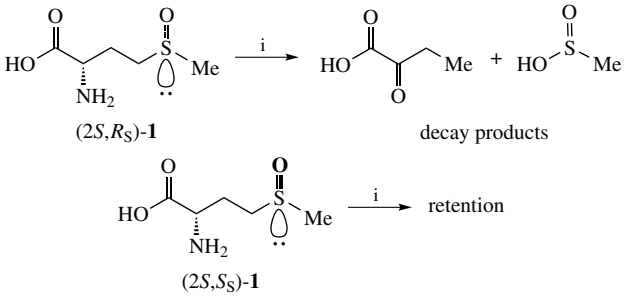
Methionine-γ-lyase (EC 4.4.1.11, MGL) used in this work belongs to the extensive family of pyridoxal-5′-phosphate (PLP) dependent lyases that have a wide substrate specificity and absolute kinetic preference toward the stereogenic center at the substrate α-carbon atom. It catalyzes the reversible α,γ-elimination of L-methionine and its analogues, *S*-alkyl-L-methionines, as well as β-elimination of L-cysteine and analogues to give ammonia, α-keto acids and thiols.[17] It was revealed that MGL also catalyzed the α,β-elimination of L-cysteine *S*-alk(en)yl sulfoxides to give thiosulfinates that were found to have *in vitro* antimicrobial activity against gram-negative and gram-positive bacteria.[18] The mutant form of the recombinant enzyme from *Citrobacterfreundii* with replacement of histidine by Cys115 cleaves sulfoxides more efficiently than wild-type MGL.[19]

Studies of the reaction of the enzyme with (2*S*,S_S) and (2*S*,R_S) L-cysteine *S*-alk(en)yl sulfoxides disclosed that C115H MGL decomposed both diastereomers to an equal extent,[20] *i.e.*, that process was nonselective with respect to the configuration of the leaving group in the β-elimination reaction. Similar experiments with α,γ-elimination were not carried out before, and the question of the kinetic preference of the enzyme toward a stereogenic center of a certain configuration in the γ-leaving group remained open thus far.

Our goal was to explore the reaction of MGL with a new substrate, *i.e.*, a diastereomeric mixture of (2*S*,S_S)- and (2*S*,R_S)-(*S*)-methionine *S*-oxide **1**. This is important because MGL is considered as a promising anticancer agent. The defect of metabolism known as the 'methionine dependence' is the inability of tumor cells to synthesize methionine.[21] To date, the antitumor efficiency of the enzyme has been shown *in vitro* and *in vivo*.[22,23]

The experiments were carried out at room temperature by taking aliquots of the reaction mixture at certain time intervals.[†] An accurately weighed amount of the standard (*R*)-Phe was added to each aliquot and the amount of each stereoisomer in the reaction mixture was determined. The experimental results and chromatographic analysis of the reaction mixtures demonstrated that the concentration of the major (2*S*,S_S) diastereomer did not change in the course of the reaction with MGL, whereas the concentration of the minor (2*S*,R_S) diastereomer gradually decreased (Table 1). Accordingly, the ratio of diastereomers also changed from 63 : 37 to 84 : 16 during the reaction time studied.

[†] The enzymatic process was carried out in a solution of potassium phosphate buffer (10 ml, pH 8.1) containing pyridoxal phosphate (1 mmol), L-methionine sulfoxide (40 mg, 0.24 mmol), and methionine-γ-lyase (39 mg) at room temperature with stirring. During the reaction, aliquots (0.2 ml) of the reaction mixture were taken at regular intervals, in which the relative content of diastereomers was determined. For this purpose, standard (*R*)-Phe solution (0.2 ml, 0.24 mmol) was added to each aliquot, and the mixture was refluxed at 100 °C for 15 min, separated from the protein precipitate and evaporated. The residue was analyzed by NMR combined with HPLC on a chiral column (see Online Supplementary Materials). The reaction products were further analyzed by IR spectroscopy and mass spectrometry.



Scheme 1 *Reagents and conditions*: i, methionine-γ-lyase, phosphate buffer (pH 8.1), room temperature, 24 h.

Table 1 Action of methionine-γ-lyase on (*S*)-methionine *S*-oxide **1** S_S/R_S diastereomeric mixture.

t/h	HPLC data/[a] mmol (%)	
	(2*S*,S_S)-**1**	(2*S*,R_S)-**1**
0	15.1 (63)	8.9 (37)
5	16.2 (74)	5.8 (26)
24	14.4 (84)	2.8 (16)

[a] Chirobitic TAG column 250×4.6 mm; MeOH/H_2O (60 : 40) + 0.2% DEA, 0.70 ml min^{-1}, 210 nm UV detector.

Thus, the enzyme catalyzes the stereoselective cleavage of only one of the stereoisomers in the diastereomeric mixture, while the other one remains unchanged. The latter was isolated after 48 h processing with *ee* > 92%. Such a high stereoselectivity of this enzyme toward the configuration of the chiral center on the sulfur atom in the presence of one more chiral atom at the γ-position has been discovered for the first time. All the reaction products were identified. The ^{1}H NMR spectra of the starting compound containing a superposition of the spectra of the two stereoisomers are transformed on treatment with MGL into distinct spectra of the major (2*S*,S_S)-**1** diastereomer. The positive sign specific rotation values of the original stereoisomer mixture ($[\alpha]_D^{20} = +3.74$) remains positive ($[\alpha]_D = +2.02$) in the final reaction mixture. The decomposition products of the minor diastereomer include α-ketobutyric and methanesulfinic acids identified by IR, ^{1}H NMR spectroscopy and mass spectrometry. Thus, the IR spectrum of the product mixture isolated after the reaction contains, in addition to the signals of the pure diastereomer, also intense bands at 1090 and 980 cm^{-1} characteristic of methanesulfinic acid, as well as signals of the methylthio group at 1317 cm^{-1}. New bands corresponding to the carbonyl group of α-ketobutyric acid appear at 1630 and 1600 cm^{-1}. The mass spectral decomposition of the mixture is also consistent with the presence of an (*S*)-methionine *S*-oxide stereoisomer, α-ketobutyric and methanesulfinic acids. MALDI data (103.9545) additionally indicate the presence of ketobutyric acid. The formation of sulfinic acid in the reaction products can be explained by the autoxidation of monomethyl sulfoxide, which is formed as an intermediate upon decomposition.

The mechanism of elimination reactions catalyzed by MGL implies proton transfer to the leaving group as one of the stages. It was suggested according to X-ray diffraction analysis,[24] that side group of active site Lys (Lys210 of *C. freundii* MGL) is the proton donor in the case of α,β-elimination reaction of sulfur containing amino acids catalyzed by the PLP-dependent enzymes, whereas active site Tyr (Tyr113 of *C. freundii* MGL) is the donor in α,γ-elimination reaction. These groups are located on the opposite sides of the cofactor's pyridoxal ring plane. Thus, the chiral environment of the leaving group should principally differ in α,β- and α,γ-elimination reactions. This may have been the reason for the differences in the enzyme stereoselectivity toward the chiral leaving groups in the reactions in question.

The use of wild-type MGL allowed us to isolate one of the stereoisomers by a relatively rare approach utilizing the high enzyme selectivity toward one of the substrate's diastereomers. As a result, MGL consumes only (2*S*,R_S)-**1** diastereomer as the substrate in the γ-elimination reaction while the other one remains unchanged. This allows one to isolate the major (2*S*,S_S)-**1** diastereomer with high enantiomeric purity.

Enzymatic technique for the preparation of chiral sulfoxides that is enantio-complementary to the known (*S*)-enatiomer-reducing activity of methionine sulfoxide reductase A was described previously.[25] Kinetic resolution has been successfully accomplished with high enantioselectivity, and (*R*)-configured chiral sulfoxides were prepared in ~50% yield.[26] Such examples of the separation of diastereomers due to their different resistance to external effects, including chiral ones to which enzymes may be attributed,[1] are quite rare. In fact, diastereomeric ferrocene[27] and paracyclophane[28] complexes were previously separated due to their different stability on the carrier surface, which resulted in the complete decomposition of one of the diastereomers by silica gel chromatography and isolation of pure stereomeric products in each of the examples studied.

It is of note that such a high stereoselectivity of this enzyme to the configuration of the chiral center on the γ-sulfur atom in the presence of one more chiral atom at the α-position was observed for the first time. This fact expands the prospects of MGL utilization in processes involving the isolation of individual stereoisomers of physiologically active compounds with central chirality not only in the α-position of amino acids[12,13] but also additionally in the γ-position.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.03.030.

References

1 U. T. Bornscheuer and R. J. Kazlauskas, *Hydrolases in Organic Synthesis*, 2nd edn., Wiley-VCH, Weinheim, 2006.
2 P.-Y. Stergiou, A. Foukis, M. Filippou, M. Koukouritaki, M. Parapouli, L. G. Theodorou, E. Hatziloukas, A. Afendra, A. Pandey and E. M. Papamichael, *Biotech. Adv.*, 2013, **31**, 1846.
3 A. Kamal, G. B. R. Khanna, R. Ramu and T. Krishnaji, *Tetrahedron Lett.*, 2003, **44**, 4783.
4 A. Luna, I. Alfonso and V. Gotor, *Org. Lett.*, 2002, **4**, 3627.
5 T. Sakai, Y. Miki, M. Tsuboi, H. Takeuchi, T. Ema, K. Uneyama and M. Utaka, *J. Org. Chem.*, 2000, **65**, 2740.
6 R. Liu, P. Berglund and H.-E. Hogberg, *Tetrahedron: Asymmetry*, 2005, **16**, 2607.
7 P. Borowiecki and M. Bretner, *Tetrahedron: Asymmetry*, 2013, **24**, 925.
8 O. M. Rodrigo, A. de Souza, L. M. C. Matos, K. M. Gonzalves, I. C. R. Costa, I. Babics, S. G. F. Leite, E. G. Oestreicher and O. A. C. Antunesa, *Tetrahedron Lett.*, 2009, **50**, 2017.
9 F. M. Plieva, K. A. Kochetkov, I. Singh, V. S. Parmar, Yu. N. Belokon' and V. I. Lozinsky, *Biotechnol. Lett.*, 2000, **22**, 551.
10 E. A. Markvicheva, S. V. Kuptsova, T. Yu. Mareeva, A. A. Vikhrov, T. N. Dugina, S. M. Strukova, Yu. N. Belokon, K. A. Kochetkov, E. N. Baranova, V. P. Zubov, D. Poncelet, V. S. Parmar, R. Kumar and L. D. Rumsh, *Appl. Biochem. Biotechnol.*, 2000, **88**, 145.

11 K. A. Kochetkov, M. A. Galkina and O. M. Galkin, *Mendeleev Commun*., 2010, **20**, 314.
12 K. V. Alferov, N. G. Faleev, E. N. Khurs, Yu. N. Zhukov and R. M. Khomutov, *Mendeleev Commun.*, 2002, **12**, 2.
13 K. V. Alferov, N. G. Faleev, E. N. Khurs, Yu. N. Zhukov and R. M. Khomutov, *Mendeleev Commun.*, 2003, **13**, 127.
14 O. O. Kolodiazhna, A. O. Kolodiazhna and O. I. Kolodiazhnyi, *Tetrahedron: Asymmetry*, 2013, **24**, 37.
15 S. Gogoi and N. P. Argade, *Tetrahedron: Asymmetry*, 2006, **17**, 927.
16 T. Saravanan, R. Selvakumar, M. Doble and A. Chadha, *Tetrahedron: Asymmetry*, 2012, **23**, 1360.
17 H. Tanaka, N. Esaki and K. Soda, *Enzyme Microb. Technol*., 1985, **7**, 530.
18 E. A. Morozova, V. V. Kulikova, A. N. Rodionov, S. V. Revtovich, N. V. Anufrieva and T. V. Demidkina, *Biochimie*, 2016, **128**–**129**, 92.
19 V. V. Kulikova, N. V. Anufrieva, S. V. Revtovich, A. S. Chernov, G. B. Telegin, E. A. Morozova and T. V. Demidkina, *IUBMB Life*, 2016, **68**, 830.
20 V. Kulikova, E. Morozova, A. Rodionov, V. Koval, N. Anufrieva, S. Revtovich and T. Demidkina, *Biochimie*, 2018, **151**, 42.
21 J. O. Mecham, D. Rowitch, C. D. Wallace, P. H. Stern and R. M. Hoffman, *Biochem. Biophys. Res. Commun.*, 1983, **117**, 429.
22 R. M. Hoffman, *Expert Opin. Biol. Ther*., 2015, **15**, 21.
23 K. Kawaguchi, Q. Han, S. Li, Y. Tan, K. Igarashi, T. Murakami, M. Unno and R. M. Hoffman, *Cells*, 2019, **8**, 410.
24 A. Messerschmidt, M. Worbs, C. Steegborn, M. C. Wahl, R. Huber, B. Laber and T. Clausen, *Biol. Chem*., 2003, **384**, 373.
25 V. Nosekand and J. Míšek, *Chem. Commun.*, 2019, **55**, 10480.
26 J. Yang, Y. Wen, L. Peng, Y. Chen, X. Cheng and Y. Chen, *Org. Biomol. Chem*., 2019, **17**, 3381.
27 V. V. Dunina, E. D. Razmyslova, O. N. Gorunova, M. V. Livantsov and Y. K. Grishin, *Tetrahedron: Asymmetry*, 2005, **16**, 817.
28 V. V. Dunina, E. I. Turubanova, M. V. Livantsov, K. A. Lyssenko, N. V. Vorontsova, D. Yu. Antonov and Y. K. Grishin, *Tetrahedron: Asymmetry*, 2009, **20**, 1661.

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