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Molecular Structure and Spectral Properties of Methionine Sulfone, Product of Methionine Oxidation

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Abstract—Methionine sulfone , a product of complete oxidation of sulfur in methionine amino acid, was subjected to X-ray diffraction study on a single crystal. According to X-ray diffraction data the packing of methionine sulfone molecules in a crystal is characteristically strongly anisotropic and it is stabilized by a network of intermolecular hydrogen bonds.

Methionine sulfone [2-amino-4-(*S*-methylsulfonyl)butanoic acid, $MetSO_2$] is a product of complete oxidation of sulfur in a natural amino acid methionine. In the natural objects the rests of methionine sulfone are present as postsynthetic modifications in the active centers of some enzymes [1]. They also arise at deep oxidative damage of proteins. Unlike the product of partial methionine oxidation, methionine sulfoxide, the methionine sulfone both as an amino acid and a protein unit cannot be efficiently reduced into methionine by the enzymatic systems in the living organism. The formation of methionine sulfone units in proteins is also considered to be irreversible. The free methionine sulfone is an inhibitor of several enzymes from glutamine metabolism [2, 3]. The L-$MetSO_2$ is used in biochemistry as a specific inhibitor of glutaminesynthesase enzyme (EC 6.3.1.2) [3]. This property is caused presumably by similarity of methionine sulfone and the transition state in the reaction of glutamine formation.

Methionine sulfone is also interesting as an object for study of involvement of its different donor centers into coordination of various metals. The most significant is the interaction of amino acids with antitumor platinum complexes in whose metabolism methionine derivatives play important role as show the latest data [4, 5]. The sulfur atom in methionine sulfone does not possess donor qualities, therefore $MetSO_2$ is the closest analog of methionine incapable of coordination at the sulfur atom. The reactions of different nucleophiles with platinum complexes we have studied with the use of optically active sulfoxide marker [6, 7]. Since to the natural methionine and its oxidation products the optical activity is inherent and it may hamper the application of the above method we have carried out a comparative analysis of properties of the oxidized methionine forms. We subjected methionine sulfone to the X-ray diffraction analysis. Although quite a number of X-ray data on methionine and its derivatives [9–14 and others] are known, the high-resolution structural analysis of methionine sulfone, the product of complete sulfur oxidation, was performed by us for the first time. The analysis revealed a number of untypical features of the molecular packing of these amino acid characterized by a lot of intermolecular hydrogen bonds (IHB).

In the study were used commercial amino acids after checking their purity by NMR spectroscopy. Main parameters of ^1H and ^{13}C NMR spectra of methionine (L-MetH), products of its incomplete (L-MetSO) and complete (L-$MetSO_2$) oxidation are presented in Table 1. ^1H NMR spectra of methionine derivatives under study have characteristic for amino acids pattern complicated by typical nonequivalence of proton pairs in the groups β-CH_2 and γ-CH_2 that becomes obvious at recording spectra on NMR spectrometer with higher operating frequency.

The chemical shift of protons of groups γ-CH_2 and SCH_3 is definitely affected by the extent of sulfur oxidation. The NMR and circular dichroism parameters of L-MetH within error limits coincide with those

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Table 1. Parameters of NMR and circular dichroism (CD) spectra for oxidized methionine forms

Compound	^{1}H, δ, ppm[a]				^{13}C, δ, ppm					CD (pH 7.0), Δε, l mol^{-1} cm^{-1}, [λ, nm]
	$C^{\alpha}H$	$C^{\beta}H_2$	$C^{\gamma}H_2$	SCH_3	COO	C^{α}	C^{β}	C^{γ}	SCH_3	
L-MetH	3.84 m	2.14 m	2.61 m	2.10 s	175.5	55.3	31.0[b]	30.2[b]	15.3	+1.3 [197]
L-MetSO[c]	3.86 m	2.27 m	3.40 m	2.72 s	174.5	54.8	37.9	49.6	25.1	–0.11 [225]
						54.7	38.0			+1.6 [199]
L-MetSO$_2$	3.89 m	2.36 m	3.41 m	3.13 s	174.1	54.3	41.1	51.3	24.3	+1.8 [198]

[a] The chemical shift given corresponds to the center of a multiplet; the chemical shifts of nonequivalent proton pairs at C^{β} and C^{γ} were not calculated.

[b] Assignment of C^{β} and C^{γ} signals in L-Met spectrum is tentative.

[c] Mixture of diastereomers with different configuration of sulfoxide group.

contained in the spectral database [8]. The attempt of quantitative analysis of multiplet signals for coupling constants evaluation and estimation of $MetSO_2$ structural parameters in solution gave ambiguous results, presumably due to the additional complication of signals because of the presence of conformers. In the ^{13}C NMR spectra of L-MetSO as a rule appear signals from two diastereomers distinguished by different absolute configuration of the sulfoxide group.

The natural methionine exists exclusively as L-isomer (in *S*-configuration), and it possesses optical activity. The main chromophore in the free methionine molecule is the carboxy group appearing in the circular dichroism (CD) spectra as a band at ~200 nm that is characteristic at all the α-amino acids. Optical activity of the group results in a medium CD ($\Delta\varepsilon \approx$ 1...2 l mol^{-1} cm^{-1}) in the region of 200 nm for α-amino acids exempt of the other chromophores. In most cases the positive sign of the band corresponds to L-isomer (note that the sign of the band not always is the same as the sign of the optical rotation $[\alpha]_D$). The intensity of the effect and the position of the band maximum strongly depend on the charges of the carboxy and amino groups and consequently on pH. The same band (with retention of the sign) is observed also in the oxidized derivatives of L-methionine (Table 1). In the methionine spectrum in the neutral medium also can be observed weak induced optical activity of sulfur atom transitions. The intensity of CD effect grows insignificantly with methionine sulfur oxidation.

Methionine sulfone readily forms crystals of trigonal symmetry on evaporating water solutions. Since the structural data on these compounds were lacking one of the crystals of $MetSO_2$ we subjected to X-ray diffraction study. The molecular configuration and numbering of atoms are given on Fig. 1, the principal structural parameters are listed in Table 2.

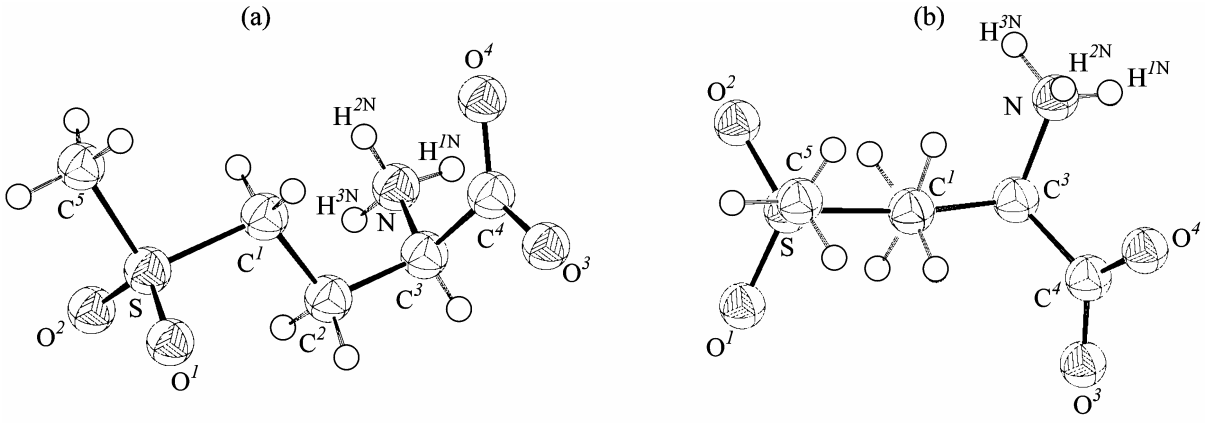

Fig. 1. Structure of methionine sulfone (D-enatiomer configuration is presented, the ellipsoids correspond to 50% probability). (a) General view, (b) view along C^1–C^2 bond

Table 2. Principal structural parameters of optically pure methionine sulfone[a]

Bond	*l*, Å	Angle	deg
		Sulfonyl group	
S–O^2	1.4414 (12)	O^1SO^2	116.94 (8)
S–O^1	1.4450 (11)	O^1SC^1	108.33 (7)
S–C^5	1.7528 (15)	O^1SC^5	107.76 (8)
S–C^1	1.7696 (13)	O^2SC^1	108.93 (8)
		O^2SC^5	109.77 (8)
		C^5SC^1	104.38 (7)
		Aminocarboxymethyl group	
O^3–C^4	1.2482 (15)	$O^3C^4O^4$	125.96 (11)
O^4–C^4	1.2527 (15)	$O^3C^4C^3$	117.13 (11)
C^3–C^4	1.5343 (15)	$O^4C^4C^3$	116.85 (10)
N–C^3	1.4884 (15)	NC^3C^2	111.00 (9)
		NC^3C^4	109.70 (9)
		$NC^3C^4O^4$	23.80 (3)
		Methyene group	
C^1–C^2	1.5301 (17)	SC^1C^2	110.05 (8)
C^2–C^3	1.5302 (17)	$C^1C^2C^3$	113.17 (10)
		$C^2C^3C^4$	111.10 (9)
		$C^5SC^1C^2$	–176.58
		$SC^1C^2C^3$	(13)
		$C^1C^2C^3C^4$	172.51 (11)
			54.79 (17)

[a] Atoms are numbered as on Fig. 1.

The crystal is built up from methionine sulfone molecules in zwitter-ionic form. All molecules in the crystal are of the same absolute configuration. The solution of the X-ray data of the crystal under study in the direct method revealed the absolute *R*-configuration of methionine sulfone (D-enentiomer). This fact was unexpected since the initial commercial preparation contained predominantly L-isomer (of optical purity over 90%) as was shown unambiguously by the positive sign of the CD band at ~200 nm of the water solution of the original commercial compound. All the crystals of $MetSO_2$ from different series were visually of a trigonal symmetry, and the solutions of chosen at random 20 crystals possessed a positive CD band at ~200 nm. Thus every crystal of $MetSO_2$ obtained from the preparation used contained only the molecules of a single enantiomer. Apparently the discrepancy in the CD and X-ray analysis data are due to insufficient accuracy in determination of the absolute configuration from the available experimental data. The specific feature of the diffraction pattern obtained is small amount of information with respect to the absolute configuration, and despite the high resolution (R_1 0.0202) the parameter of the «absolute» configuration is large, 055 (5) evidencing the low reliability of absolute configuration found. The other reason may be partial separation of enantiomers of racemic $MeSO_2$ during crystallization. The latter assumption can be easily rationalized proceeding from the specific packing of methionine sulfone molecules that will be treated below. Then the crystal subjected to the X-ray analysis was actually built up of D-enantiomeric molecules. In both instances the molecular properties and crystal lattice of the D-enantiomer would be common to the natural L-enantiomer whose structure and crystal lattice can be obtained by inversion operation: *–x, –y, –z*. The bond lengths and bond angles in both enetiomers and parameters of hydrogen bonds therein are obviously the same.

Molecular skeleton C^5–S–C^1–C^2–C^3 has unstrained conformation and is nearly planar. The sulfonyl and aminocarboxymethyl groups are located *anti* with respect to C^β–C^γ bond (C^1–C^2; Fig. 1b). Similar conformation was found in a related compound, (2*S*, S*S*)-sulfoximine [9]. This conformation corresponds to maximal distance between the negatively charged carbonyl group and the substituents attached to sulfur having effective negative charge: the molecule is maximally extended in length. For unoxidized methionine *gauche* conformation at C^β–C^γ bond is more common [9, 10]. However in proteins are found also other forms, including eclipsed conformation. In solutions of methionine sulfone the *anti* and *gauche* conformations apparently coexist as rotamers. This assumption allows understanding of excessively complicated multiplet pattern of the signals in the NMR spectra from protons attached to C^β (C^2) and C^γ (C^1).

The structural parameters of D-methionine sulfone are the most similar to those of (2*S*, S*S*)-methionine sufoximine [9]. However in contrast to the methionine sulfoximine, and also to the other related molecules: methionine (L-enantiomer and racemic) [10, 11], acetyl methionine [12], *N*-formylmethionine [13], S-methylmethioninesulfonium cation [14], and norleucine [10], the methionine sulfone is packed into unusual anisotropic crystal lattice stabilized by IHB where all three protons of NH_3^+ group and both oxygens of COO^- are involved. The packing of molecules in the crystal is shown on Fig. 2, parameters of the

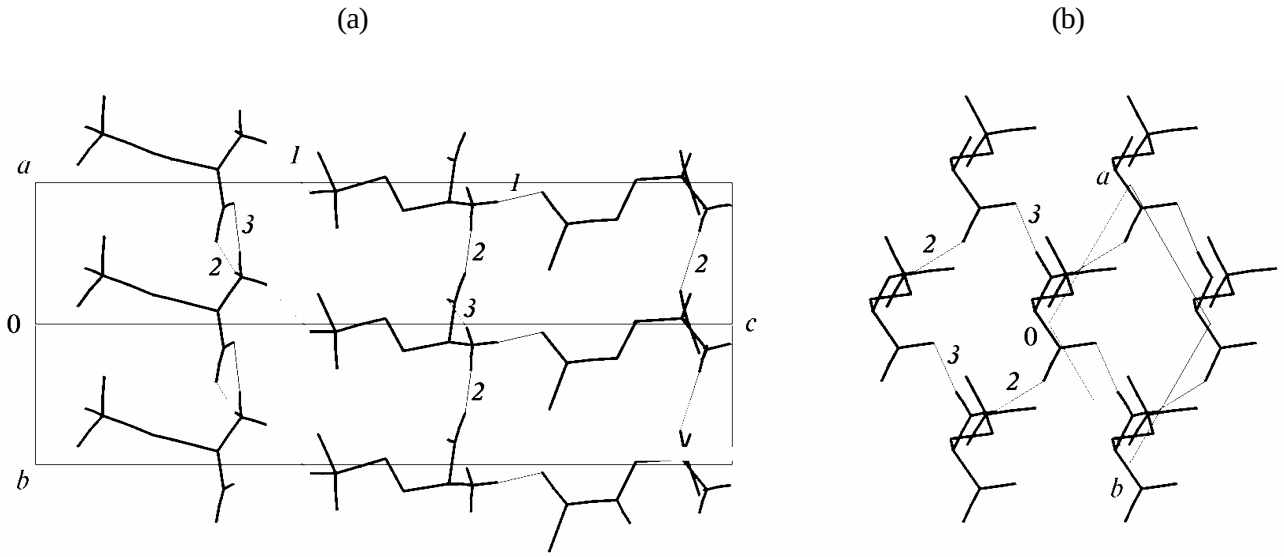

Fig. 2. Packing of molecules of methionine sulfone. Hydrogen bonds are drawn by dashed lines. Hydrogen atoms not involved in hydrogen bond formation are not shown. On Fig. 2b only one molecule from each unit cell is presented. Hydrogen bonds are numbered as in Table 3.

hydrogen bonds are given in Table 3. The long axes of molecules in the crystal lattice are located at a small angle to the third order screw axis *c*, and in the crystal thus arise tightly packed oriented layers of molecules perpendicular to the said screw axis. The inner part of the layer consists of nonpolar methylene groups, one surface is formed of charged carboxy and amino groups, the other one of polar sulfonyl groups. The packing of molecules in the layer is stabilized by a network of hydrogen bonds where are involved two of the three bonds formed by the protons of NH_3^+ group (Fig. 2b).

One more, less classic hydrogen bond, is formed by third proton of NH_3^+ group and the oxygen atom of the sulfonyl group belonging to a molecule from the neighbor layer thus binding together the layers in the crystal (Fig. 2a).

Geometrical parameters of the molecule have typical values, and their deviations from statistical mean values are due to hydrogen bonds formation (packing effect). The sulfur atom possesses tetrahedral configuration distorted by reciprocal repulsion of oxygen atoms. O^1SO^2 angle rightfully most strongly deviates from tetrahedral angle and amounts to 116.94 (8) deg (120.3 deg in the sulfoximine). The bond lengths S–O^2 1.4414 (12) and S–O^1 1.4450 (11) Å correspond to statistical mean bond lengths in sulfones. Bond lengths sulfur-oxygen and sulfur-carbon C^5–S 1.7528 (15) and C^1–S 1.7696 (13) Å in the methionine sulfone are shorter than in methionine sulfoxide, and the distortion of the tetrahedral configuration is less pronounced. Published quantum-chemical calculations for methionine sulfone in the basis 6-31G* [15] verestimate the length of C–S bond (1.775 and 5 Å) and underestimate the length of S–O bond (1.438 Å). The carboxy group is virtually planar, small difference in the length of O^3–C^4 è O^4–C^4 bonds and their value indicate the charged state of the group. A slight elongation of the O^4–C^4 bond as compared

Table 3. Parameters of intermolecular hydrogen bonds in a crystal of optically pure methionine sulfone

Hydrogen bond	Donor	Acceptor	N–H, Å	H···O, Å	N···O, Å	NHO angle, deg	Symmetry operation
1	$N–H^{1N}$	O_2	0.85	2.25	2.987 (3)	147	$-x + y, -x, z + 1/3$
2	$N–H^{2N}$	O_3	0.81	2.04	2.8266 (19)	160	$x, y + 1, z$
3	$N–H^{3N}$	O_4	0.91	1.77	2.7191 (15)	172	$x + 1, y + 1, z$

with O^3–C^4 bond may be ascribed to involvement of O^4 in tight hydrogen bond. The main distinction from the canonical structure of α-amino acids is deviation of nitrogen atom from the carboxy group plane formed by atoms C^3, C^4, O^3 and O^4 by ~0.55 Å: In crystal structures of the majority of amino acids this value is close to zero [10, 13]. It should be noted once more that the above described structure consists of molecules of a single enantiomer. It is presumable that lattice formation with such numerous IHB is possible only with the molecules of the same absolute configuration, and the probable symmetric packing containing racemic mixture of enantiomers is liable to be less favorable. Therefore the special features of $MetSO_2$ molecules packing amply explain both separation of enantiomers by crystallization and the uncommon trigonal symmetry of crystals. This effect can be applied to preparation of small amounts of $MetSO_2$ of 100% optical purity from the trigonal single crystals. This difference also can be attributed to the participation of NH_3^+ and COO^- groups in the hydrogen bonds: Formation of the IHB in the lattice of $MetSO_2$ governed by symmetry requirements turned out to be more feasible than location of the nitrogen atom in the carboxy group plane (corresponding to the strongest intramolecular electrostatic attraction of NH_3^+ and COO^- groups).

EXPERIMENTAL

Amino acids used in the study were commercial products: L-methionine (Merck, optical purity over 95%), L-methionine sulfoxide [a mixture of (2*S,SS*) and (2*S, SR*) diastereomers] and L-methionine sulfone of Hungarian production (optical purity with respect to the α-amino group over 90%). Methionine sulfone was purified by recrystallization from water.

NMR spectra of saturated solutions in D_2O were recorded in standard 5 mm NMR tubes on spectrometers Bruker AC-200 (^{1}H 200.13, ^{13}C 50.33 MHz) and Bruker AM-500 (^{1}H 500.13 MHz). Final resolution of ^{1}H NMR spectra was 0.61 Hz (100 scans, 8K points). Resolution of ^{13}C NMR spectra was 1.4 Hz (data acquisition time 0.74 s, 10000 scans, 8K points). All results are given in ppm with respect to TMS. No additional references were used, the chemical shifts were measured relative to the signal of the deuterated solvent. The validity of assignment of ^{1}H and ^{13}C signals in the spectra of methionine sulfone was confirmed by recording DEPT spectra and by two-dimensional correlation method XHCORR (^{1}H–^{13}C).

CD spectra in the region 190-240 nm were recorded on automatic dichrograph Mark-V and spectropolarimeter Cary-60 with a device for measuring CD. The CD spectra of amino acids solutions in distilled water were run in cylindrical cells with light path of 1 and 0.1 cm.

For X-ray diffraction study was selected a colorless single crystal of plate form, crystal habit $0.50 \times 0.47 \times 0.02$ mm obtained by crystallization from water solution. The measurements were performed at 20°C on automatic diffractometer Enraf-Nonius CAD-4 by θ/2θ scanning with the use of K_α radiation of wave length 0.71069 Å, Θ_{max} 39.85° filtered through Nb filter. Measurement range by indices $0 \le h \le 8$, $0 \le k \le 7$, $-40 \le l \le 41$, 1511 reflections were analyzed with $I > 2s(I)$, $F(000)$ 288. The intensities of 3 standard reflections were measured every 60 min, and they remained stable within ±3%. The correction for absorption of the X-rays by the sample was done with a factor 3.93 cm^{-1}. Trigonal crystals, a 5.353 (10), c 22.963 (4) Å, V 569.8 (18) Å^3, ρ_{calc} 1.584 g cm^{-3}, space group $P3_2$, Z 3. The structure was solved by the direct method and refined in the full-matrix anisotropic approximation for F^2, positions of hydrogen atoms were obtained by the difference synthesis and refined in isotropic approximation. Extremum values of the final difference function were 0.306 and –0.182 eÅ^{-3}. The values of R-factors were [$I \ge 2\sigma(I)$]: R_1 0.0202, wR_2 0.0548. Parameter of the «absolute» configuration was 0.55 (5).

REFERENCES

1. Gouet, P., Jouve, H.M., and Dideberg, O., *J. Mol. Biol.,* 1995, vol. 249, p. 933.
2. Arad, G. and Kulka, R.G., *Biochim. Biophys. Acta.*, 1978, vol. 544, 153.
3. Brenchley, J.E., *J. Bacteriol*., 1973, vol. 114, p. 666.
4. Reedijk, J., *Chem. Rev*., 1999, vol. 99, p. 2499.
5. Reedijk, J. and Teuben, J.-M., *J. Bioinorg. Chem*., 2000, vol. 5, p. 463.
6. Lobadyuk, V.I., Spevak, V.N., and Skvortsov, N.K., *Zh. Obshch. Khim*., 1996, vol. 66, 871.
7. Skvortsov, A.N., de Vekki, D.A., Stash, A.I., Belsky, V.K., Spevak, V.N. and Skvortsov, N.K., *Tetrahedron: Asymmetry*, 2002, vol. 13, p. 1663.

8. *Spectral Database SDBS* (30.11.2002) http:www.aist.go.jp/RIODB/SDBS/
9. Chevrier, B., Moras, D., Jeannoda, V.L.R., Creppy, E.E., and Dirheimer, G., *Acta Cryst*., 1986, vol. C42, p. 1632.
10. Torii, K. and Iitaka, Y., *Acta Cryst*., 1973, vol. V29, p. 2799.
11. Mathieson, A. McL., *Acta Cryst*., 1952, vol. 5, p. 332.
12. Ponnuswamy, M.N. and Trotter, J., *Acta Cryst*., 1985, vol. C41, 917.
13. San Chen, C. and Partharathy, R., *Acta Cryst.,* 1977, vol. B33, p. 3332.
14. Del Re, G., Gavuzzo, E., Giglio, E., Lelj, F., Mazza, F., and Zappia, V., *Acta Cryst*., 1977, B33, 3289.
15. Borisov, Yu.A., Zolotarev, Yu.A., Laskatelev, E.V., and Myasoedov, N.F., *Izv. Akad. Nauk, Ser. Khim.*, 1998, 1487.