A phosphinic analogue of methionine is a substrate of L-methionine-γ-lyase and induces the synthesis of the enzyme in *Citrobacter intermedius* cells

Kirill V. Alferov,[a] Nikolai G. Faleev,[b] Elena N. Khurs,[a] Yurii N. Zhukov[a] and Radii M. Khomutov*[a]

[a] *V. A. Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 119991 Moscow, Russian Federation. Fax: +7 095 135 1405; e-mail: khomutov@genome.eimb.relarn.ru*

[b] *A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, 119991 Moscow, Russian Federation. Fax: +7 095 135 5085; e-mail: ngfal@ineos.ac.ru*

10.1070/MC2002v012n01ABEH001550

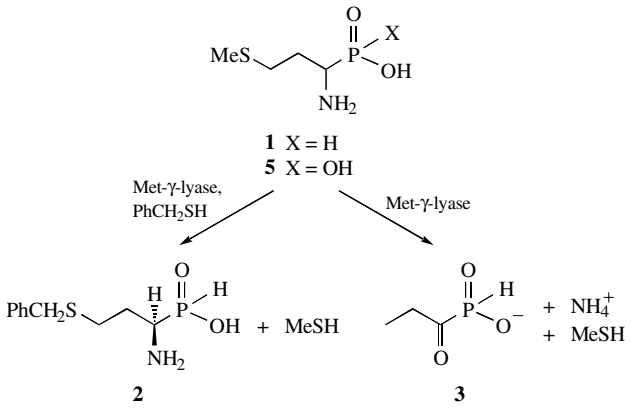
1-Amino-3-(methylthio)propylphosphinic acid likewise methionine, is a substrate in α,γ-elimination and γ-substitution reactions catalysed by pyridoxal-5′-phosphate-dependent L-methionine-γ-lyase and is capable to induce the synthesis of this enzyme in *Citrobacter intermedius* cells as does L-methionine.

Among organophosphorus analogues of amino acids, biologically active compounds were found whose activity is determined by competition with natural amino acids and their ability to participate in amino acid metabolism. 1-Amino-3-(methylthio)propylphosphinic acid **1**, which is an analogue of methionine, is an interesting compound of this kind. It displays high antimicrobial activity[1] and suppresses the growth of malignant tumors.[2] The origin of the biological activity of **1** remains unclear, although it is known that compound **1** can affect protein biosynthesis at the stage of methionyl-t-RNA formation[3] and, probably, biomethylation processes as a result of its conversion into an analogue of *S*-adenosylmethionine.[2] On the other hand, the influence of **1** on pyridoxal-5′-phosphate (PLP)-dependent enzymes was not studied. These enzymes play an important role in the metabolism of sulfur-containing amino acids; consequently, the effects on them should be taken into consideration in the studies of the biological activity of **1**.

In this work, we examined the interaction of **1** with PLP-dependent L-methionine-γ-lyase [L-methionine-methanethiol lyase (deaminating) E.C. 4.4.1.11] from *C. intermedius*. This enzyme is a typical representative of α,γ-eliminating lyases, which catalyses decomposition of L-methionine to methanethiol, α-ketobutyrate and the ammonium ion. Methionine-γ-lyase is contained in many microorganisms, and the pathogenicity of some of them is associated with the activity of this enzyme.[4] Methionine-γ-lyase displays broad specificity with respect to substrate structures and types of chemical reactions;[5] however, its interaction with organophosphorus substrate analogues and related compounds was not studied.

We found that compound **1** is a substrate in α,γ-elimination and γ-substitution reactions catalysed by methionine-γ-lyase, and it induces formation of this enzyme in *C. intermedius* cells.

Methionine-γ-lyase from *C. intermedius* catalyses the stereospecific formation of *S*-alkylhomocysteins from L-methionine and thiols.[6] We found that compound **1** is a substrate in the reaction of γ-substitution, and its interaction with benzylthiol in the presence of methionine-γ-lyase affords optically active 1-amino-3-(benzylthio)propylphosphinic acid **2** (a phosphinic analogue of *S*-benzylhomocysteine) in 12% yield (Scheme 1).† The optical activity of compound **2** indicates that γ-substitution is enantioselective as it is in the case of the natural substrate.‡ This opens new possibilities for the synthesis of optically active phosphinic analogues of sulfur-containing amino acids that otherwise may be prepared only by very complicated methods.



Scheme 1

We found that amino acid **1** is a substrate in α,γ-elimination reaction catalysed by methionine-γ-lyase,§ and it is decomposed to 1-oxopropylphosphinic acid **3** (a phosphinic analogue of α-ketobutyrate) (Scheme 1), which was identified as 2,4-dinitrophenylhydrazone **4**.¶ The values of K_m for compound **1** (1.25 mM) and L-methionine (1.13 mM) are almost equal but taking into account that only one enantiomer of racemic **1** is transformed in the reaction the real K_m value is two times lower (0.625 mM). Judging from these values, the affinity of **1** to the enzyme is comparable to the affinity of the natural substrate. Thus, the obvious difference between structural parameters of H(HO)(O)P- and HOOC- groups has no effect at the stage of Michaelis complex formation. At the same time, the value of k_{cat} for **1** was lower than that for L-methionine by a factor of 35.

† The cells of *Citrobacter intermedius* AKU-10 containing methionine-γ-lyase were grown according to published procedure.[6]

Racemic 1-amino-3-methylthiopropylphosphinic and phosphonic acids (**1** and **3**) were synthesised according to the published procedure.[7]

Benzylthiole (0.5 ml) and frozen cells of *Citrobacter intermedius* (0.5 g) were added to a solution of **1** (169 mg, 1 mmol) in 20 ml of a 0.1 M potassium phosphate buffer solution containing 0.1 mM PLP. The reaction mixture was stirred on a shaker for five days at 25 °C. The protein was denatured by adding 30% trichloroacetic acid (1 ml) and removed by centrifugation. The solvent was evaporated *in vacuo*, the residue was dissolved in water (1 ml) and applied to a 40 ml column with Dowex 50x8 (H^+ form). The column was washed with water (100 ml), and product **2** was eluted with a 5% ammonia solution. The fractions containing **2** were evaporated *in vacuo* to dryness. The residue was dried *in vacuo* over P_2O_5 to give phosphinic analogue **2** (30 mg, 12%), mp 221 °C, $[\alpha]_D^{20}$ –16.3° (0.5% in 1 M HCl). R_f 0.61 (Pr^iOH–25% NH_4OH–H_2O, 7:1:2); R_f 0.46 (Bu^nOH–AcOH–H_2O, 12:3:5). ^1H NMR (0.25 M NaOD in D_2O) δ: 1.63–2.05 (m, 2H, CH_2CH), 2.53–2.79 (m, 3H, SCH_2CH_2 and CH), 3.80 (s, 2H, CH_2Ph), 6.72 (dd, 1H, PH, *J* 486 Hz, *J* 1.8 Hz), 7.40 (s, 5H, Ph). Found (%): C, 48.68; H, 6.31; N 5.41. Calc. for $C_{10}H_{16}NO_2PS$ (%): C, 48.97; H, 6.57; N, 5.71.

‡ The stereochemistry of this reaction most likely is the same as for the natural substrate because no reasons for its changing are evident. Note that the absolute stereospecificity of another PLP-dependent lyase, tyrosine plenol-lyase, was not changed on going from the natural substrate to its phosphinic analogue.[8]

§ The cell extract containing methionine-γ-lyase was prepared from *C. Intermedius* cells according to published procedure.[6] Protamine sulfate as a 5% solution was added to the extract in an amount equal to 5% of the total protein amount. The precipitate formed was separated by centrifugation, the solution was kept at 60 °C for 5 min, and the denatured protein was separated by centrifugation. The activity of the preparation was assayed by measuring the rate of α-ketobutyrate formation from L-methionine according to Friedemann.[9] One unit of enzymic activity was determined as the enzyme amount catalysing the transformation of 1 μmol of L-methionine per minute at 30 °C and a concentration of L-methionine equal to 40 mM.

Thus, the enzyme is specific to the structure of the acid fragment at the intermediate stages of substrate transformation. Note that 1-amino-3-methylthiopropylphosphonic acid **5** (a phosphonic analogue of methionine), which is different from compound **1** only by an additional HO group at the phosphorus atom, is not a substrate of the enzyme, although the structural parameters of the phosphorus-containing fragment remained almost unchanged. These data suggest that the biological activity of acid **1** can be associated with PLP-dependent transformation into 1-oxopropylphosphinic acid, which is an organophosphorus analogue of α-keto-acids known as effective inhibitors of thiaminepyrophosphate-dependent transformations.

We also examined the properties of compound **1** *in vivo*, *i.e.*, with respect to *C. intermedius* culture cells. We found that **1** had almost no effect on cell growth; however, it can penetrate into the cells like L-methionine and induce the biosynthesis of methionine-γ-lyase when the cells were grown in a synthetic medium containing acid **1** instead of L-methionine.†† However, these properties were not found in acid **5**; this is most probably due to the well-known problem of aminophosphonate transport through the cell wall. It is noteworthy that regulatory activity of this kind was not observed previously at the cell level in organophosphorus analogues of amino acids. With respect to the biological activity of acid **1**, a principally new possibility exists to affect the metabolism of amino acids by influencing the biosynthesis of enzymes of this metabolic pathway.

Thus, compound **1** can be considered as a competitive inhibitor of the PLP-dependent enzyme, which is capable to undergo slow substrate transformations to form new organophosphorus compounds. It acts as an inductor of the biosynthesis of the enzyme similarly to the natural amino acid.

This work was supported by the Russian Foundation for Basic Research (grant nos. 00-15-97844, 00-04-48242 and 01-04-48636).

References

1 J. G. Dingwall, in *Proc. III Int. Conf. Chem. Biotech. Biol. Active Comps*., Sofia, Bulgaria, 1985, vol. 1, p. 87.
2 R. M. Khomutov, Yu. N. Zhukov, A. R. Khomutov, E. N. Khurs, D. L. Kramer, J. T. Miller and K. V. Porter, *Bioorg. Khim*., 2000, **26**, 735 (*Russ. J. Bioorg. Chem*., 2000, **26**, 647).
3 A. I. Biryukov, T. I. Osipova and R. M. Khomutov, *FEBS Lett*., 1978, **91**, 246.
4 M. Yoshimura, Y. Nakano, Y. Yamashita, T. Oho, T. Saito and T. Koga, *Infect. Immun*., 2000, **68**, 6912.
5 H. Tanaka, N. Esaki and K. Soda, *Biochemistry*, 1977, **16**, 100.
6 N. G. Faleev, M. V. Troitskaya, V. S. Ivoilov, V. V. Karpova and V. M. Belikov, *Prikl. Biokhim. Mikrobiol*., 1994, **30**, 458 (in Russian).
7 T. I. Osipova, A. R. Khomutov, Yu. N. Zhukov and R. M. Khomutov, *Izv. Akad. Nauk, Ser. Khim*., 1999, 1360 (*Russ. Chem. Bull*., 1999, **48**, 1348).
8 N. G. Faleev, Yu. N. Zhukov, E. N. Khurs, O. I. Gogoleva, M. V. Barbolina, N. P. Bazhulina, V. M. Belikov, T. V. Demidkina and R. M. Khomutov, *Eur. J. Biochem*., 2000, **267**, 6897.
9 F. Friedemann and G. Haugen, *Z. Biol. Chem*., 1943, **177**, 415.

Received: 9th January 2002; Com. 02/1876

¶ Amino acid **1** (169 mg, 1 mmol) was dissolved in 15 ml of 0.1 M potassium phosphate buffer (pH 8.0), which contained 2 μmol of pyridoxalphosphate and 0.22 U ml^{-1} of methionine-γ-lyase. The reaction mixture was allowed to stand in the dark at 30 °C for 72 h. A 30% aqueous CCl_3COOH solution (25 ml) was added to the reaction mixture, and the precipitate formed was separated by centrifugation. The resulting solution was treated with an excess of 2,4-dinitrophenylhydrazine in 2 M HCl for 2 h at 25 °C, and a mixture of hydrazone **4** and 2,4-dinitrophenylhydrazine was extracted with EtOAc. Compound **4** was isolated from an EtOAc solution by extraction with 0.1 M potassium phosphate buffer (pH 8.0). Extracts containing **4** were combined, acidified with an aqueous 10 M HCl solution (5 ml) and acid **4** was extracted with EtOAc. The solvent was evaporated, the residue was washed with Et_2O and dried *in vacuo* over P_2O_5/KOH to give 15 mg (5.5%) of hydrazone **4** (a mixture of *syn*- and *anti*-isomers). R_f 0.69 (Bu^iOH–H_2O–EtOH, 5:4:2). ^{1}H NMR (CD_3OD–D_2O, 3:2) δ: 1.07–1.20 (m, 3H, *syn*- and *anti*-*Me*CH_2), 2.98 and 3.24 (2q, 2H, *syn*- and *anti*-MeCH_2, *J* 7.5 Hz), 6.99 (d, 1H, PH, *J* 751 Hz), 7.83 and 7.91 [2d, H(6), *syn*- and *anti*-$C_6H_3N_2O_4$, *J* 9 Hz], 8.15 and 8.23 [2d, H(5), *syn*- and *anti*-$C_6H_3N_2O_4$, *J* 9 Hz], 8.85 and 8.88 [2s, H(3), *syn*- and *anti*- $C_6H_3N_2O_4$]. UV, λ_{max}/nm (1.6 M NaOH): 546 (6340).

†† The specific activity of methionine-γ-lyase in *C. intermedius* cells grown in a synthetic medium containing acid **1** as an inductor was found to be 0.5 mU mg^{-1} of protein.