Stable organophosphorus analogues of *S*-adenosylmethionine and *S*-methylmethionine

Kirill V. Alferov, Yurii N. Zhukov, Elena N. Khurs and Radii M. Khomutov*

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

10.1070/MC2003v013n06ABEH001856

The organophosphorus analogues of the biologically significant sulfonium compounds *S*-adenosylmethionine and *S*-methylmethionine are much more stable than their carboxylic prototypes.

S-Adenosylmethionine [Met(Ado)] is of significance for cellular metabolism; it participates in important reactions such as biomethylation and the synthesis of polyamines. In various cells under pathological conditions considerable differences in these processes have been observed. This allows us to consider the metabolism of Met(Ado) as a perspective biochemical target while searching for new physiologically active compounds and antimicrobial medicines. *S*-Methylmethionine (vitamin U), another natural sulfonium compound, is also a donor of methyl groups, although of lesser importance than Met(Ado).

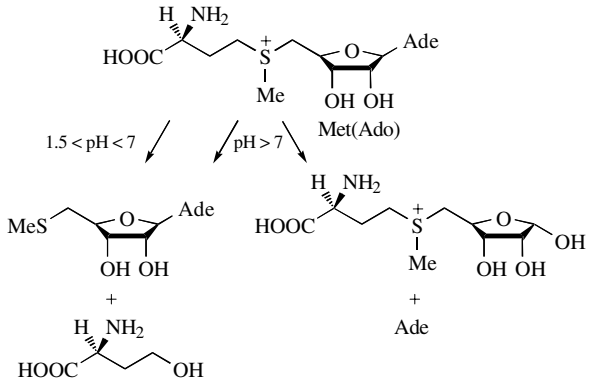
The analogues of Met(Ado) obtained to the present time mostly contain modified nucleoside moieties and have low affinities to corresponding enzymes, while analogues with modified amino acid moieties remain almost unstudied.[1] These analogues, as Met(Ado) itself, are unstable at neutral pH values, which is caused by the presence of a reactive sulfonium moiety and an appropriately placed carboxyl group within the same molecule. Therefore, the analogues of Met(Ado) are not usually considered as effective tools for affecting Met(Ado)-dependent biomethylation processes, taking into account the transport of charged compounds into the cell.

In this respect, organophosphorus analogues of Met(Ado), in which the carboxyl group is replaced by an acidic phosphorus-containing fragment, appeared promising. It was shown that a phosphinic analogue of Met(Ado), 5′-{*S*-[3-amino-3-(hydroxyphosphoryl)propyl]-*S*-methylthionia}-5′-deoxyadenosine chloride **1** (Scheme 1), possesses high affinity to tRNA-methyltransferase and is a substrate of this enzyme[2] (*i.e.* it can participate in biomethylation reactions). Compound **1** is an effective inhibitor of Met(Ado)-decarboxylase, a key enzyme of polyamine biosynthesis, while the C–P bond of compound **1** remained stable to the action of the enzyme.[2] Moreover, acid **1** displays biolological activity and suppresses the growth of tumor cells L1210 affecting the intracellular level of Met(Ado).[3] When cells were incubated with 1-amino-3-(methylthio)propylphosphinic acid **2**, the inhibition of the cell growth and formation and the intracellular accumulation of analogue **1** were observed. This observation opens new possibilities to affect the Met(Ado) metabolism.[3] Thus, a comparative study of the stability of analogue **1** and related compounds seemed appropriate, allowing us, in terms of reactivity, to estimate qualitative differences between the organophosphorus analogues of sulfonium compounds and their natural prototypes.

Note that the phosphonic analogue of methionine [in which the carboxylic group is replaced by the $P(O)(OH)_2$ group] did almost not affect the growth of tumor cells, as well as a phosphonic analogue of Met(Ado) (5′-{*S*-[3-amino-3-(dihydroxyphosphoryl)propyl]-*S*-methylthionia}-5′-deoxyadenosine acetate **3**). At the same time, it was shown recently that a phosphonic analogue of *S*-adenosylhomocysteine, the product of demethylation of Met(Ado), is an effective irreversible inhibitor of *S*-adenosylhomocysteine hydrolase – the enzyme whose activity determines the level of biomethylation in the cell.[4] We studied the stability and decomposition of the phosphinic analogues of biologically significant sulfonium compounds, chloride **1** and iodide of 1-amino-3-(dimethylthionia)propylphosphinic acid **4**,[†] under different conditions.

1 R = Ado, X = Cl, Y = H
3 R = Ado, X = OAc, Y = OH
4 R = Me, X = I, Y = H
5 R = Me, X = I, Y = OH

Scheme 1



Scheme 2

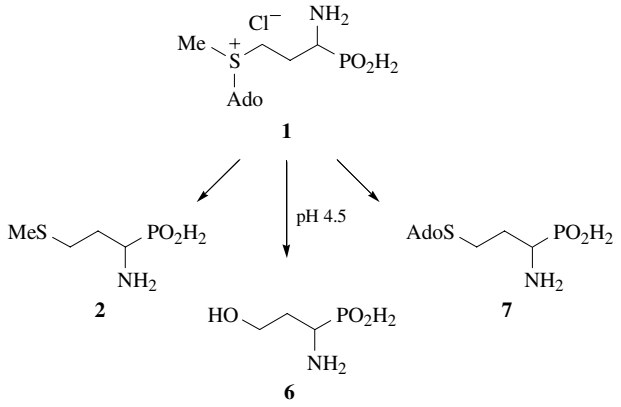
Met(Ado) is a chemically unstable compound, which decomposes in aqueous solutions at pH > 1.5 to form *S*-methylthioadenosine and homoserine according to the mechanism of intramolecular alkylation [k_H 6×10^{-6} s^{-1} (37 °C, pH 7.5) or 4×10^{-3} s^{-1} (100 °C, pH 4.5)], while at pH > 7 the hydrolysis to adenine and *S*-pentosylmethionine becomes significant [k_H 3×10^{-6} s^{-1} (37 °C, pH 7.5)] (Scheme 2).[7] Note that an important catabolic pathway of Met(Ado) in the cells catalysed by Met(Ado)-lyase consists of decomposition to *S*-methylthioadenosine and homoserine.[8]

First, we studied the stability of analogue **1** under conditions described elsewhere.[7] The progress of decomposition of acid **1** was monitored by ^1H NMR spectroscopy. Therefore, the reaction was carried out in a deuterated potassium phosphate buffer (pD 7.1, which corresponds to pH 7.5). Under these conditions, compound **1** remained stable for several days, whereas a half-decomposition period of Met(Ado) was 28 h. Moreover, acid **1** remained stable in D_2O upon the incubation of its solution for few months at ambient temperature. While boiling in water at pH 4.5, analogues **1** and **3**[‡] were found to be more stable than Met(Ado) (k_H 0.2×10^{-3} s^{-1} for compound **1**).

Thus, at pH 4.5, compound **2** and phosphinic analogues of homoserine and *S*-adenosylhomocysteine (Scheme 3, compounds **6** and **7**, respectively)[§] were found among the products of decomposition of **1** and no additional formation of adenine and a phosphinic analogue of *S*-pentosylmethionine was observed at pD 7.1 (*cf.* ref. 7).[¶]

S-Methylmethionine is more stable than Met(Ado) and decomposes with the formation of homoserine and dimethyl

[†] Acid **1** (mixture of diastereomers), racemic acids **4**, **5** and *S*-methylmethionine iodide were synthesised according to the published procedures.[5,6]



Scheme 3

sulfide only upon heating in neutral and alkaline solutions[10] (according to our data, k_H 1.65×10^{-3} s^{-1} at 100 °C in 0.5 M NaOH). Under these conditions, organophosphorus analogue **4** and 1-amino-3-(dimethylthionia)propylphosphonic acid iodide **5** were also found to be more stable (k_H 0.3×10^{-4} or 1.1×10^{-4} s^{-1}, respectively), while the main product of the transformation of compound **4** is methionine analogue **2**, whereas acid **5** gave a multicomponent mixture.†† The synthesis of homoserine analogue **6** from compound **4** was carried out only under special conditions: the heating of **4** in an AcOH–AcONa mixture followed by acidic hydrolysis.[6]

Note that *S*-adenosylhomocysteamine, which is free of the COOH group, appeares to be more stable than Met(Ado).[11] Therefore, different stability and ways of degradation of sulfonium salts **1** and **4**, as compared to their carboxylic prototypes, may be explained by the low reactivity of the mesomeric phosphinic anion in intramolecular alkylation. The transformations of this anion into ethers in case of aminoalkylphosphinates were carried out only by methylation with diazomethane.[12] Thus, the decomposition of sulfonium salts **1** and **4** proceeds without the participation of acidic moieties of the molecules. The stability of compounds **3** and **5** may be explained similarly.

Thus, the acidic phosphorus-containing group in the test analogues of natural sulfonium compounds is capable not only to carry out an 'anchor' function while binding to target enzymes, likewise the carboxyl group, but also to increase considerably the stability of the analogues, including the stability to the action of Met(Ado) catabolic enzymes.

This work was supported by the Russian Foundation for Basic Research (grant no. 03-04-48949) and a grant for Scientific School no. 1800-2003.4.

References

1 R. T. Borchardt, in *Enzymatic Basis of Detoxification*, ed. W. B. Jakoby, Academic Press, New York, 1980, vol. 2, p. 43.
2 V. I. Syrku, L. L. Zavalova and R. M. Khomutov, *Bioorg. Khim.*, 1986, **12**, 839 (in Russian).
3 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**, 718 (*Russ. J. Bioorg. Chem.*, 2000, **26**, 647).
4 J. A. Steer, P. B. Sampson and J. F. Honek, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 457.
5 Yu. N. Zhukov, A. R. Khomutov, T. I. Osipova and R. M. Khomutov, *Izv. Akad. Nauk, Ser. Khim.*, 1999, 1360 (*Russ. Chem. Bull.*, 1999, **48**, 1348).
6 K. V. Alferov, Yu. N. Zhukov, E. N. Khurs, T. I. Osipova and R. M. Khomutov, *Izv. Akad. Nauk, Ser. Khim.*, 2001, 303 (*Russ. Chem. Bull., Int. Ed.*, 2001, **50**, 316).
7 J. L. Hoffman, *Biochemistry*, 1986, **25**, 4444.
8 S. K. Shapiro and A. N. Hather, *J. Biol. Chem.*, 1958, **233**, 631.
9 N. G. Faleev, Y. 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.
10 V. N. Bukin and G. N. Khuchua, in *Vitamin U (S-metilmetionin): priroda, svoistva, primenenie* [*Vitamin U (S-methylmethionine): Origin, Properties, Applications*], eds. V. N. Bukin and V. E. Anisimov, Nauka, Moscow, 1973, p. 10 (in Russian).
11 V. Zappia, P. Galletti, A. Oliva and A. Santis, *Anal. Biochem.*, 1977, **79**, 535.
12 P. P. Gianousis and P. A. Bartlett, *J. Med. Chem.*, 1987, **30**, 1603.

Received: 2nd October 2003; Com. 03/2182

‡ A 10% solution of I_2 in EtOH was added dropwise with stirring to a mixture of acid **1** (100 mg, 0.22 mmol), aqueous 0.5 M HI (2 ml) and EtOH (1 ml) until a weak yellow colour was retained for 2–3 min. The reaction mixture was lyophilised; the residue was dissolved in water, applied onto FN-18 paper and chromatographed in the Bu^nOH–AcOH–H_2O system (12:3:5). The strip of paper containing acid **3** was cut out and eluted with water; the eluate was concentrated *in vacuo* to dryness. The residue was dried *in vacuo* over P_2O_5 to give compound **3** (64%, 69 mg): mp 168 °C, R_f 0.03 (Pr^iOH–25% NH_4OH–H_2O, 7:1:2); R_f 0.03 (Bu^nOH–AcOH–H_2O, 12:3:5). ^1H NMR (400 MHz, D_2O) δ: 2.02 (s, 3H, $MeCOO^-$), 2.07–2.54 (m, 2H, CH_2CH), 2.99 (s, 3H, MeS^+), 3.13–3.30 (m, 1H, $CHCH_2$), 3.45–3.72 (m, 2H, $^+SCH_2CH_2$), 3.84–3.99 (m, 2H, 5′-CH_2S^+), 6.04 (d, 1H, H-1′, *J* 4.7 Hz), 8.24 and 8.26 (2s, 1H, H-2 and 1H, H-8).

§ A solution of acid **1** (31 mg, 0.068 mmol) in 2 ml of a 0.05 M sodium acetate buffer (pH 4.5) was boiled for 5 h. The quantitative determination of acid **1** was performed by TLC.[9] At known time intervals, aliquot portions of the reaction mixture were applied to a Silufol plate. The plate was developed by using Bu^nOH–AcOH–H_2O (12:3:5) and/or Pr^iOH–25% NH_4OH–H_2O (7:1:2), dried and treated with a ninhydrin solution. Spots giving positive ninhydrin reactions were cut and extracted with a 0.5% $CdCl_2$ solution in 50% ethanol, and the absorbance of the extracts was determined at 505 nm. To construct the calibration curve, aliquot portions of an authentic **1** solution were subjected to the same treatment. The identity of the products of decomposition with authentic **2**, **6** and **7** was shown by TLC on a Silufol plate developed by the upper layer of the Bu^nOH–AcOH–H_2O (12:3:5) and Pr^iOH–25% NH_4OH–H_2O (7:1:2) systems. Spots were visualised by UV and colour reactions with ninhydrin and ammonium molybdate.

The rate constants k_H were obtained by fitting the data (concentrations of acid **1** *vs.* time) to the rate equation of a first-order reaction.

Taking into account a considerable difference in the decomposition rates for Met(Ado) and **1** and according to published data,[7,11] we assumed that the rate constants of hydrolysis of diastereomers of **1** are equal.

¶ A solution of acid **1** (20 mg, 0.04 mmol) in 0.7 ml of a 0.2 M deuterated potassium phosphate buffer (pD 7.1) was incubated at 37 °C for 144 h. ^1H NMR spectra were recorded at known time intervals.

†† The solutions of *S*-methylmethionine and acids **4** and **5** (40 mg) in an aqueous 0.5 M NaOH solution were boiled for 6 h. The quantitative determination of *S*-methylmethionine and acids **4** and **5** and the identification of decomposition products with authentic homoserine, compounds **2** and **6**, were carried out as described above.