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ACTION OF TRIMETHYL PHOSPHATE ON AMINO ACIDS. SELECTIVE METHYLATION OF L-CYSTEINE AND RELATED COMPOUNDS

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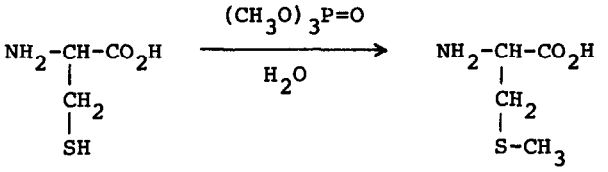
Quite recently, trimethyl phosphate (TMP) was shown to function as an alkylating agent in vivo, and when fed to mice, rats etc., this compound was rapidly metabolized to dimethyl hydrogen phosphate and S-methylcysteine was identified as an urinary metabolite.[1] Similar results were found in other trialkyl phosphate, but TMP was the only member of the series exhibitting the sterilzing activity.[2] These reports prompt us to communicate the results of invitro experiments on the action of TMP on 21 amino acids under homogeneous aqueous conditions. Our observations provide the additional evidences on selectively fast S-methylation of cysteine and a basis for a mechanistic proposal as well.

Reactions were generally carried out, using L-amino acids (10.0 mmole), TMP (20.0 mmole) and water (7 ml) at pH 5 - 11 , 37°C. The pH of the solution was maintained at the same level with the occasional addition of 0.5 N sodium hydroxide. The progress of reactions were checked by the usual quantitative ninhyrin-coloring test on the extracted aqueous solution of the spot in the paper chromatography of the reaction mixtures.

The analysis indicated the rapid and selective methylation only for cysteine to give S-methylcysteine as shown in the Figure 1. For instance, cysteine is methylated almost completely after 1.5 hr at pH 8, 37°C. On the contrary, 20 other amino acids (Gly, Ala, Val, Leu, Ileu, Phe, Ser, Thr, Lys, Arg, Asp, Asp-NH_2, Glu, Glu-NH_2, Cys-Cys, Met, Tyr, Pro, Try, His) did not form any products at all under pH 5 - 8 even after 5 hr. At the higher pH value (9 - 11), only His and Try reacted slightly to generate N^4-methyl- and



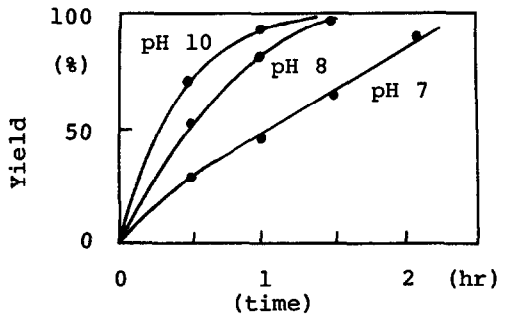


Fig. 1 The time-course of the yield of S-methylcysteine in the reaction of cysteine (10.0 mmol) and TMP (20.0 mmol) in water (7 ml) at 37°C.

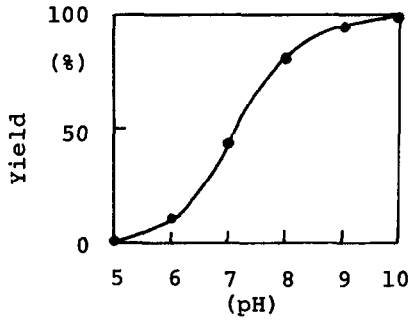


Fig. 2 The effect of pH on the yield of S-methylcysteine in the reaction of Fig. 1. (reaction time = 1 hr)

N^5-methylhistidines (both compounds in ~5 % yields) and O^{phe}-methyltyrosine (~7 %) after 3 hr.

S-Methylcysteine was isolated along with dimethyl hydrogen phosphate in the comparable yields.[3] This dimethyl ester, however, did not exhibit methylating property any longer. Similar evidences were described in our previous experiments on reactions of TMP with nucleic acid-bases.[4]

Poly-cysteine (MW 3800, 6.0 mg) also underwent methylation reaction on its sulfhydryl groups upon treatment with TMP (5.0 mmole) in water-DMF (1 : 1 v/v, 5 ml) at pH 8.5 for 5 hr. The hydrolysis of the resulting peptide with 6N hydrochloric acid (3 ml) at 100°C for 48 hr provided S-methylcysteine in the yield of 32 %.

The kinetics of the reaction of cysteine and TMP was consistent excellently with the second order equation of rate = k[Cys][TMP], giving Ea = ~17 Kcal/mol, ΔS^* = -27 eu at pH 10.05. Since the reaction occurs especially easily at the pH value (> 8) where the sulfhydryl group (pKa = 8.2 at 35°C)[5] dissociate appreciably into the mercaptide ion (RS^-) as shown in the Figure 2, the S-methylation may take place most likely via a neucleophilic attack of the

mercaptide ion toward one of three methyl groups of TMP.

From the above discussion, S-methylcysteine and dimethyl hydrogen phosphate may be considered to be formed by the bimolecular interaction of TMP with free cysteine and/or the cysteine moiety of proteins in the above mentioned in-vivo studies.[1] In the latter case, the S-methylated proteins may be metabolized subsequently to liberate S-methylcysteine. These processes might be also intermediated by enzymes.

Lastly, authors wish to mention that the above procedure could be applied generally to mercaptanes for selective and facile S-methylation; e. g. when 2-mercaptoethanol and 2-aminoethanethiol (0.05 mol) were treacted with TMP (0.05 mol) in water (5 ml) at pH 11, 37°C for 9 hr, the corresponding S-methyl derivatives were isolated in 75 % and 86 %, respectively. Benzylmercaptane and thiophenol were similarly converted to the corresponding S-methyl derivatives in 89 % and 80 %.[6]

The present results, thus, suggest also that TMP would be useful not only as a convenient methylating agent for a sulfhydryl group but also a useful modifing agent for the cysteine moiety of a protein. Other methylating agents such as methyl iodide, methyl esters of sulfurous oxy acids tend to react also at hydroxyl groups of Thy, Tyr and Ser as well as amino groups of Lys, His, etc. There are several selective alkylating agents available for cyteine such as iodoacetate, N-ethylmaleimide, etc., but they always introduce bulky alkyl group on sulfur atom.[7] The modification of proteins with TMP is now under study.

References and Footnotes

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2) S. S. Epsteine et al., Science, 168, 584 (1970); K. F. Dyer et al., Mutat. Res., 11, 327 (1972).

3) A homogeneous mixture of L-cysteine (1.21 g, 0.01 mol), TMP (2.81 g, 0.02 mol) in water (30 ml) was kept at pH 7, 37°C with occasional addition of 1.5 N sodium hydroxide. After 4 hr, the reaction mixture was concentrated and the residue was mixed with ethanol (30 ml) to precipitate

S-methylcysteine as a crude product (1.05 g, 75 %). It was crystallized from 90 % ethanol to give a pure compound (0.78 g, 56 %), mp 220-221°C (220°C by J. F. Thompson, Nature, 178, 593 (1956)). Ir and nmr spectra were identical with those of the authentic sample. $[\alpha]_D^{18} = -23.5°$ (c = 1, H_2O) (the authentic sample showed - 27.7 ° in the same condition; thus, about 7.5 % racemization occured in our procedure).

The ethanol solution above obtained was adjusted to pH 8 - 9 by aqueous sodium hydroxide and concentrated. The residue was mixed with acetone to precipitate the sodium salt of dimethyl hydrogen phosphate, 1.2 g (83 %), mp 241 - 245°C (the authentic sample, mp 246 - 248 °C).

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6) S-Methyl-2-mercaptoethanethiol was isolated by extraction of the reaction mixture with chloroform and the subsequent concentration and distillation of the organic extract, 3.41 g (75 %). Physical constants (bp, nmr and mass spectra) were consistent with the assigened structure. The O-methyl derivative was not found in the gass chromatographic analysis of the reaction mixture. Other mercaptanes described in the text were processed in the similar manner to give the corresponding S-methyl derivatives.

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