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STEREOCHEMICAL ASPECTS OF TRANSMETHYLATIONS OF POTENTIAL BIOLOGICAL INTEREST

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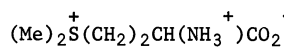
Abstract - The natural distribution and stereochemical feature of *S*-methyl-L-methionine are discussed. By means of doubly labelled substrates evidence is provided for the stereochemical course of the transmethylation reaction, catalyzed by a transferase from jack bean seeds, and proceeding with *S*-methyl-L-methionine as the donor, homocysteine as the acceptor. The pro-R methyl group of the former is transferred with a stereoselectivity of 90 % or more.

INTRODUCTION

In 1954, Shive and co-workers demonstrated the occurrence of *S*-methylmethionine (1) (MMT) in cabbage and several other vegetables.[1] Subsequently, 1 has been reported as a constituent of asparagus,[2,3] jack beans,[4] pelargonium and mint leaves,[5] green tea,[3,6] soybean meal,[7] celery,[3] tomatoes,[3,8] sweet corn,[9] potatoes,[10] apples,[11] and milk.[12] The amazingly wide distribution of 1 justifies inquiries into its chemistry, biosynthesis, metabolism, and biological significance.

Even before its discovery as a natural product, MMT (1) had been shown to support the growth of rats on a methionine-free diet,[13] a finding later modified to obtain only when cystine was present in the diet.[14]



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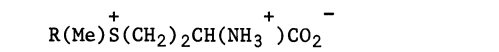


2 R = Adenosyl

Other early reports list MMT (1) as a methyl donor in the biosynthesis of creatine in rat liver slices,[15] though not in homogenates,[16] and as a methionine-replacing factor, in some cases superior to methionine itself, in a number of microorganisms.[17]

Early studies on yeast[18] and bacteria,[19] in which the combined administration of homocysteine and MMT, but neither of the two alone, was shown to support the production of S-adenosylmethionine (2) (SAM)[18] or methionine,[19] led to the working hypothesis that the enzyme-catalyzed-reaction:

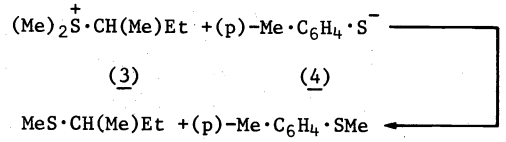
(R = L-$(CH_2)_2CH(NH_3^+)CO_2^-$)

may be operating in living cells and hence possess biological significance.[20] This assumption was subsequently substantiated through more detailed studies of the ability of several cell-free extracts to catalyze the above reaction. Thus, homocysteine methyltransferases, accepting SAM and MMT as methyl donors, have been described from rat liver,[21-23] microorganisms,[20,23-27] and plant seeds, including pea, cabbage, and jack bean.[28-30] Growing plants of the last species contain MMT,[4] rendering the methyltransferase from jack bean meal[29] particularly apposite in a functional context. This enzyme, obviously different from homocysteine methyltransferase of animal or microbiological provenance, has been purified 250-fold and accepts other donors, such as dimethylpropiothetin and SAM, the latter though with only one tenth of the activity.[29]

STEREOCHEMICAL STUDIES

The monochiral *S*-methyl-L-methionine (1) contains prochiral, diastereotopic methyl groups which must, in principle, be transferable to a chiral or achiral acceptor molecule with different rates. An exploratory study, recently conducted in our laboratory, with (RS)-2-butyl-

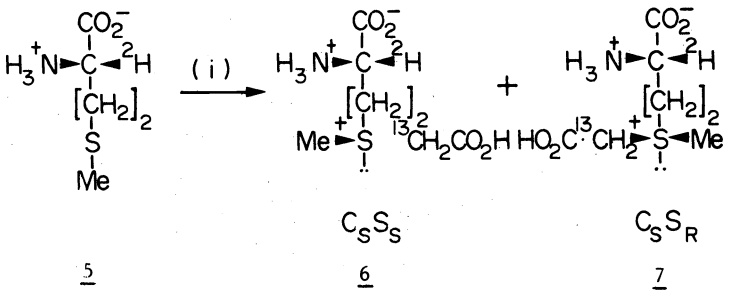
dimethylsulphonium ion (3) as the donor and 4-methylbenzenethiolate ion (4) as the acceptor molecule, revealed a virtually indiscriminative methyl group transfer insofar as a substantial, observed rate difference could be accounted for exclusively in terms of a remarkably large $^{12}C/^{14}C$-isotope effect.[31]



We have extended this stereochemical exercise to the methyl group transfer from *S*-methyl-L-methionine to homocysteine,[29] catalyzed by the enzyme from jack bean meal; we report our results in the sequel.

Synthesis of Enzyme Substrates

α-^{2}H-L-Methionine (5) (⩾95 % ^{2}H), produced by resolution[32] of the corresponding racemate (Stohler Isotope Chem. Inc.), was converted, upon treatment with 2-^{13}C-enriched bromoacetic acid (Stohler Isotope Chem. Inc.), into an approximately 1:1 mixture of diastereomeric salts of *S*-(carboxy-^{13}methyl)-α-^{2}H-L-methionine, (6) and (7) (Fig. 1.) Separation of these was accomplished by recrystallization of their polyiodides, essentially following the procedure recently described by Cornforth et al.[33] The least soluble of these gives a mono-2,4,6-trinitrobenzenesulphonate (mono-TNBS-salt) which, on analytical amino acid chromatography, possesses the longest retention time.[33,34] By x-ray diffraction, this isomer was recently demonstrated to possess the C_SS_R-configuration (7) in its cationic moiety.[33] It was found convenient in our hands to convert the two diastereomeric polyiodides into the corresponding, sparingly soluble bis-TNBS salts of (6) and (7). By ^{1}H 270 MHz n.m.r. analysis the ^{13}C-enrichment in 6 and 7 was found to be about 79 % and 73 %, respectively; analytical amino acid chromatography revealed a content of about 7 % of 7 in 6, and about 3 % of 6 in 7, both analyzed as bis-TNBS-salts; these values, however, are to be taken as only approximate.



5 6 7

Fig. 1. (i) Br^{13}CH$_2$.CO$_2$H(70.3 % ^{13}C); (α-^{2}H-L-methionine (⩾95 % ^{2}H) (985 mg), H$_2$O (7 ml); 42 h, 20°. H$_2$O ad 40 ml; KI(1.20g), KI$_3$ (1.0 M, 3.3 ml); 72 h at 4°; 5 x KI$_3$ (1.0 M, 0.66 ml) in the course of 48 h; filtration; 2.34 g (84.5 %) (least soluble isomer) (C_SS_R)[33] (7). Filtrate: KI$_3$ (1 M, 1.0 ml); 12 h at 4°; 3 x KI$_3$ (1.0 M, 1.0 ml) in the course of 8 h; filtration; 1.15 g (41.4 %) (most soluble isomer) (C_SS_S)[33] (6).

The anhydrous bis-TNBS-salts of 6 and 7 were subsequently subjected to decarboxylation as outlined in Fig. 2. Reaction conditions were mild enough to exclude significant epimerization due to pyramidal inversion of the sulphonium center. The resulting C_SS_R- and C_SS_S-*S*-(^{13}C-methyl)-α-^{2}H-methionine diastereomers, (8) and (9), were converted into their crystalline bis-TNBS-salts through a series of ion-exchange operations. ^{1}H n.m.r. analyses revealed ^{13}C contents of 69 % in 8, 76 % in 9; the specimen of 8 contained about 3.5-4 % of 9, that of 9 about 4.5-5 % of 8. Before being subjected to enzymic transfer reactions, the ions (8) and (9) were converted into their monochlorides, again by ion exchange column technique.

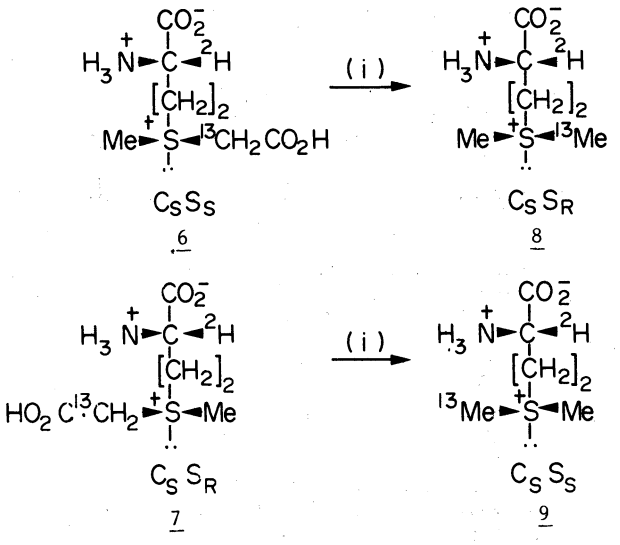


Fig. 2. (i) Anhydrous bis-TNBS-salt (400 mg), HMPTA (3 ml), 20°, 1 h; addition of pyridine (1.2 ml), 60°, 15-17 min; ion-exchange (IR-120/Na^+), elut. with NH_3; (a) HCl (b) TNBSH, $4H_2O$, 0°; 8 (222 mg, 53 %); 9 (202 mg, 48 %).

The monochlorides of 8 and 9, respectively, served as substrates for the transmethylation to homocysteine, catalyzed by an enzyme preparation from jack bean meal, prepared and partially purified according to literature directions.[29]

Enzymic Transmethylation

Incubations were set up as specified in Fig. 3, resulting in the consumption of more than 90 % of the substrates according to n.m.r. analysis of suitable model compounds; the methionine produced was isolated by ion exchange technique and subsequently converted into its bis-trimethylsilyl (bis-TMS) derivatives upon reaction with N-(trimethylsilyl) diethylamine. Control experiments, in which the substrates, or homocysteine, were omitted from the incubations, gave no trace of methionine. A non-catalyzed methionine formation, detectable in the absence of the enzyme preparation, proceeded with a rate too low to seriously compete with the enzyme-catalyzed reaction.

Reagents		Concentration
R1:	(RS)-Homocysteine lactone, hydroiodide	30.1 µmol/100 µl
R2:	8, or 9, as monochlorides	2.43 µmol/50 µl
R3:	7.5 M NaOH	1500 µmol/200 µl
R4:	1 M KH_2PO_4	2000 µmol/2000 µl
R5:	Enzyme solution	

Fig. 3. (i) R1 + R3, N_2, 20°, 5 min; R4 added. (ii) To this solution (575 µl), addition of R2 (50 µl) and R5 (625 µl), N_2, 38-39°, 200 min.

The bis-TMS derivatives of methionine, arising from enzymic transmethylation of the diastereomeric substrates, (8) and (9), Fig. 4, were separately analyzed by GLC-MS-technique.

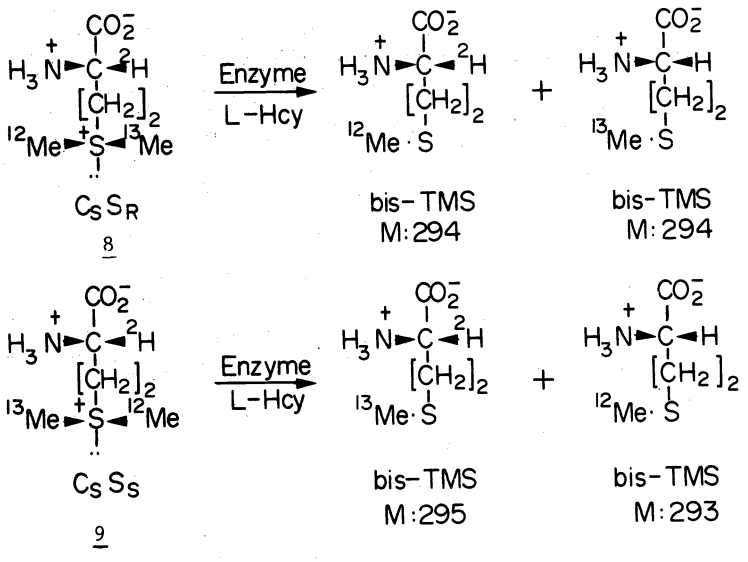
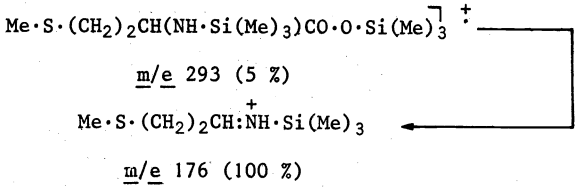


Fig. 4. Enzymic transfer of a methyl group from diastereomeric *S*-^{13}C-methyl-α-^{2}H-L-methionines to L-homocysteine.

Mass Spectrometric Analysis

The mass spectrum of the bis-TMS-derivative of methionine exhibits a molecular ion at m/e 293 with an intensity of about 5 % of that of the base peak at m/e 176, the latter arising by fission of the C_1-C_2-bond:

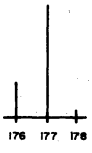


The bis-TMS-derivatives of α-^{2}H-methionine, ^{13}C-methyl-methionine, and ^{13}C-methyl-α-^{2}H-methionine gave the expected mass spectra, with the same isotope ratios in the m/e 177 and 178 region as in the m/e 294 and 295 region. Consequently, secondary isotope effects in the fragmentation can be neglected and MS-analyses can confidently be conducted on the high-intensity fragment ions at m/e 176, 177 and 178.

All bis-TMS derivatives were introduced into the mass spectrometer through a gas chromatograph with fast, repetitive scanning. Intensity determinations were performed by integration over 45 individual recordings along the gaschromatographic profile in order to compensate for an observable tendency to separation of the various isotopic species. The measured intensities, corrected for natural abundances, are schematically reproduced in Fig. 5.

In order to express the intensities in terms of per cent stereoselectivity, the relations in Fig. 6 were derived for the bis-TMS methionine fragment ions arising from the methionine produced in the enzyme-catalyzed transfer from (8) and its congeners (2x denotes the fraction, in per cent, of (8) donating the ^{13}Me group). A similar scheme can easily be derived for the diastereomer (9).

When the enrichment factors for ^{2}H and ^{13}C are designated h and c, respectively, the three equations (A)-(C) in Fig. 7 obtain. From any two of these, the sum h + c can be expressed as shown and assumes the value 1.62 on insertion of the relative intensities (cf. Fig. 5) for the C_SS_R-diastereomer (8). From the known value, 0.95, for h, a ^{13}C enrichment factor of 0.67 follows. Inserting these values in any one of the equations (A)-(C), one finds a value for 2x of 90.3. It was previously established, however, that the C_SS_R-isomer (8) employed contained about 5 % of the C_SS_S-isomer (9). When corrected for this content, the value of 2x increases to 95. Analogous calculations, performed on the isomer (9), gave the pleasingly consistent value 2x = 95.

Molecular Ions m/e	Fragment Ions m/e	% Intensity of Fragment Ions* From C_SS_R(8) m/e	From C_SS_S(9) m/e
293	176	22.1	47.6
294	177	74.9	22.2
295	178	3.0	30.2
			

*corrected for natural abundance contributions

Fig. 5. Fragment ion intensities from enzymically produced bis-TMS derivatives of methionine.

Reactants		%		Fragment Ions m/e
HCy-(H)[a] + $^{13}C^{12}C$(D)[b]	⟶	x[c]	^{13}C(H)	177
		50-x	^{12}C(H)	176
		x	^{12}C(D)	177
		50-x	^{13}C(D)	178
HCy-(H) + $^{12}C^{12}C$(D)	⟶	50	^{12}C(H)	176
		50	^{12}C(D)	177
HCy-(H) + $^{13}C^{12}C$(H)	⟶	x	^{13}C(H)	177
		50-x	^{12}C(H)	176
		x	^{12}C(H)	176
		50-x	^{13}C(H)	177
HCy-(H) + $^{12}C^{12}C$(H)	⟶	100	^{12}C(H)	176

[a]Denotes homocysteine; [b]denotes MMT with specified isotope contents in the two methyl groups and at the α-carbon; [c]2x is the fraction, in per cent, of (8), denoting the ^{13}Me group.

Fig. 6. Distribution of the bis-TMS fragment ions arising from L-methionine produced in the enzymically catalyzed transfer from C_SS_R-*S*-(^{13}methyl)-α-^{2}H-methionine (8), and its congeners, to homocysteine.

(A) $\% I^{176} = -hcx + 50\,hc - 50h - 50c + 100$
(B) $\% I^{177} = 2hcx - 100\,hc + 50h + 50c$
(C) $\% I^{178} = -hcx + 50\,hc$

It follows that:

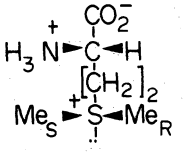
$$h + c = \frac{100 + (I^{178} - I^{176})}{50}, \quad \text{or} \quad \frac{I^{177} + 2 \cdot I^{178}}{50} \quad \text{or}$$

$$\frac{200 - (2 \cdot I^{176} + I^{177})}{50} = 1.62$$

$h = 0.95$; it follows that $c = 0.67$
From (A), (B), or (C): $2x = 90.3\ \%$

Fig. 7. Calculation of the 2x value for the enzymic transmethylation to homocysteine from the diastereomerically homogeneous C_SS_R-*S*-(^{13}methyl)-α-^{2}H-methionine (8).

The excellent agreement in the two experimentally independent series permits the conclusions: (i) that primary isotope effects can be neglected, and (ii) that the pro-R methyl group in MMT (10) is preferentially transferred to homocysteine in the enzyme-catalyzed reaction. It is noteworthy that SAM, possessing the C_SS_S-configuration (11),[33] contains its methyl group in a spatial position different from that of the pro-R methyl group in MMT (10). The way now seems open, utilizing methyl-labelled SAM, to clarify the stereochemical course of the *S*-methylation of methionine, a reaction believed to represent the biosynthesis of MMT in jack beans.[4]



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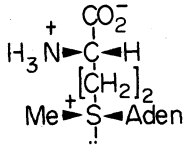

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The observed stereoselectivity, defined as $2x-(100-2x)/2x + (100-2x) = 4x-100$, thus amounts to 90 %. Its derivation from 100 % may conceivably be due to a slight competition from a non-specific, non-enzymatic transmethylation reaction, or to the presence of a few per cent of the D-enantiomer in the α-^{2}H-L-methionine utilized as the starting material. A slight epimerization of the sensitive sulphonium ion through thermal, pyramidal inversion during the various manipulations would likewise result in a diminished stereoselectivity and cannot be entirely excluded.

CONCLUSION

The work described in this lecture was designed to answer the questions: are the two methyl groups in MMT being transferred to homocysteine with different rates in the enzymically catalyzed reaction, and, if so, which methyl group is preferentially, or exclusively, transferred? The answer to the first question is that the selectivity is 90 % or better; to the second, that the pro-R methyl group in MMT is preferentially, or perhaps even exclusively, transferred. A similar approach should prove useful in clarifying other stereochemical problems associated with biological transmethylation reactions. Such problems are currently under investigation.

Acknowledgements

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REFERENCES

1. R.A.McRorie, G.L.Sutherland, M.S.Lewis, A.D.Barton, M.G.Glazener and W.Shive, J. Amer. Chem. Soc. 76 (1954) 115.
2. F.Challenger and B.J.Hayward, Chem. & Ind. (London) (1954) 729
3. F.I.Skodak, F.F.Wong and L.M.White, Anal. Biochem. 13 (1965) 568.
4. R.C.Greene and N.B.Davis, Biochim. Biophys. Acta 43 (1960) 360.
5. L.Peyron, Bull. Soc. Franc. Physiol. Vegetale 7 (1961) 46; C.A. 56, 12014b.
6. T.Kiribuchi and T.Yamanishi, Agr. Biol. Chem. 27 (1963) 56.
7. T.Hino, A.Kimizuka, K.Ito and T.Ogasawara, Nippon Nogei Kagaku Kaishi, 36 (1962) 413; C.A. 61, 10889h.
8. F.F.Wong and J.F.Carson, J. Agr. Food Chem. 14 (1966) 247.
9. D.D.Bills and T.W.Keenan, J. Agr. Food Chem. 16 (1968) 643.
10. G.Werner, R.Hossli and H.Neukom, Lebensm.-Wiss.u.Technol. 2 (1969) 145.
11. A.H.Baur and S.F.Yang, Phytochemistry 11 (1972) 2503.
12. T.W.Keenan and R.C.Lindsay, J. Diary Sci. 51 (1968) 112; C.A. 68, 38277n.
13. M.A.Bennett, J. Biol. Chem. 141 (1941) 573.
14. J.Stekol, in 'A Symposium on Amino Acid Metabolism', The Johns Hopkins Press, Baltimore Md., U.S.A. 1955, p.509.
15. P.Handler and M.L.C.Bernheim, J. Biol. Chem. 150 (1943) 335.
16. S.Cohen, J. Biol. Chem. 201 (1953) 93.
17. R.A.McRorie, M.R.Glazener, C.G.Skinner and W.Shive, J. Biol. Chem. 211 (1954) 489.
18. F.Schlenk and R.F.DePalma, Arch. Biochem, Biophys. 57 (1955) 266.
19. S.K. Shapiro, Biochim. Biophys. Acta 18 (1955) 134.
20. S.K.Shapiro, J. Bacteriol. 72 (1956) 730.
21. G.A.Maw, Biochem. J. 63 (1956) 116.
22. G.A.Maw, Biochem. J. 70 (1958) 168.
23. S.K.Shapiro and D.A.Yphantis, Biochim. Biophys. Acta 36 (1959) 241.
24. S.K.Shapiro, Biochim. Biophys. Acta 29 (1958) 405.
25. S.K.Shapiro, J. Bacteriol. 83 (1962) 169.
26. S.K.Shapiro, D.A.Yphantis and A.Almenas, J. Biol. Chem. 239 (1964) 1551.
27. E.Balish and S.K.Shapiro, Arch. Biochem. Biophys. 119 (1967) 62.
28. J.E.Turner and S.K.Shapiro, Biochim. Biophys. Acta 51 (1961) 581.
29. L.Abrahamson and S.K.Shapiro, Arch. Biochem. Biophys. 109 (1965) 376.
30. W.A.Dodd and E.A.Cossins, Biochim. Biophys. Acta 201 (1970) 461.
31. G.Grue-Sørensen, A.Kjær, R.Norrestam and E. Wieczorkowska, Acta Chem. Scand. B31 (1977) 859.
32. G.P.Wheeler and A.W.Ingersoll, J. Amer. Chem. Soc. 73 (1951) 4604.
33. J.W.Cornforth, S.A.Reichard, P.Talalay, H.L.Carrell and J.P.Glusker, J. Amer. Chem. Soc. 99 (1977) 7292.
34. H.G.Gundlach, S.Moore and W.H.Stein, J. Biol. Chem. 234 (1959) 1761.