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Biosynthesis and metabolism of 9-[5′-deoxy-5′-(methylthio)-β-D-xylofuranosyl]adenine, a novel natural analogue of methylthioadenosine

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The biosynthesis of 9-[5′-deoxy-5′-(methylthio)-β-D-xylofuranosyl]adenine (xylosyl-MTA), a naturally occurring analogue of 5′-deoxy-5′-methylthioadenosine (MTA) recently characterized, was studied in the nudibranch mollusc *Doris verrucosa*. Experiments performed *in vivo* with putative labelled precursors such as [8-^{14}C]adenine, [*Me*-^{14}C]methionine and [*Me*-^{14}C]MTA indicate that xylosyl-MTA originates from MTA. Experiments with MTA double-labelled at critical positions are consistent with a 3′-isomerization of the nucleoside through the formation of a 3′-oxo intermediate. In addition, experiments with the newly synthesized [3′-^{3}H]xylosyl-MTA are indicative for a very low turnover rate of this molecule, which therefore accumulates in the mollusc.

INTRODUCTION

5′-Deoxy-5′-methylthioadenosine (MTA) is a sulphur-containing nucleoside, ubiquitously distributed in micromolar amounts in Nature, originating from *S*-adenosylmethionine (AdoMet) metabolism through several metabolic pathways (Zappia *et al.*, 1980*a*; Williams-Ashman *et al.*, 1982; Pegg & Williams-Ashman, 1986; Della Ragione *et al.*, 1989). In mammalian tissues the spermidine synthase and spermine synthase reactions represent the quantitatively most important routes of MTA formation. The thioether exerts a significant inhibition *in vitro* on several enzymic systems: among them, the inhibition of spermine synthase is probably the most important (Pajula & Raina, 1979). Moreover, the nucleoside inhibits cell proliferation in many biological systems (Pegg *et al.*, 1981; Di Fiore *et al.*, 1984). In spite of the occurrence of multiple biosynthetic pathways, the intracellular concentration of MTA is remarkably low when compared with those of spermidine, spermine and AdoMet (Hibasami *et al.*, 1980; Seidenfeld *et al.*, 1980; Della Ragione *et al.*, 1981; Bachrach *et al.*, 1982). Indeed, the thioether does not accumulate intracellularly because of its rapid cleavage by MTA phosphorylase into adenine and 5-methylthioribose 1-phosphate, which are in turn salvaged into adenine nucleotides and methionine respectively (Williams-Ashman *et al.*, 1982; Della Ragione *et al.*, 1986, 1989).

9-[5′-Deoxy-5′-(methylthio)-β-D-xylofuranosyl]-adenine (xylosyl-MTA) represents the first described naturally occurring analogue of MTA: it has been isolated from the Mediterranean nudibranch mollusc *Doris verrucosa* and it has been identified and characterized by high-resolution m.s. and ^{1}H-n.m.r. spectrometry (Cimino *et al.*, 1986). In this molecule the ribofuranosyl moiety of MTA is replaced by a xylofuranosyl residue (Fig. 1).

The cellular content of xylosyl-MTA, measured by a reversed-phase h.p.l.c. method, ranges from 330 to 380 nmol/g of wet tissue and is two orders of magnitude higher than that of MTA (Porcelli *et al.*, 1988*b*).

The natural occurrence of xylosyl-MTA poses intriguing problems concerning its biosynthesis and physiological role.

Assuming that the xylosyl thioether does not derive directly from a dietary source, three hypotheses concerning its biogenesis can be formulated: (i) the molecule could originate from the xylofuranosyl analogue of AdoMet through the known enzymic reactions involved in MTA biosynthesis; (ii) MTA could be enzymically isomerized at the 3′-position; (iii) an independent biosynthetic pathway, unrelated to AdoMet and its metabolites, could be operative.

In the present paper experimental evidence is presented supporting the second hypothesis, as well as data elucidating the mechanism of the reaction. There is a lack of recognition of this molecule by the enzymes of MTA metabolism and it has a very low turnover rate, and therefore xylosyl-MTA accumulates in the mollusc.

A preliminary report of this work has been presented (Porcelli *et al.*, 1989).

MATERIALS AND METHODS

Chemicals

As commercial AdoMet is usually contaminated with MTA and other impurities, the sulphonium compound was routinely prepared from cultures of *Saccharomyces cerevisiae* (Schlenk & De Palma, 1957) and isolated by

Abbreviations used: MTA, 5′-deoxy-5′-methylthioadenosine; xylosyl-MTA, 9-[5′-deoxy-5′-(methylthio)-β-D-xylofuranosyl]adenine; AdoMet, *S*-adenosylmethionine; compound I, 9-[5′-deoxy-5′-(methylthio)-β-D-*erythro*-pentofuran-3′-ulosyl]adenine; compound II, 9-[5′-deoxy-5′-(methylthio)-2′-*O*-t-butyldimethylsilyl-β-D-*erythro*-pentofuran-3′-ulosyl]adenine.

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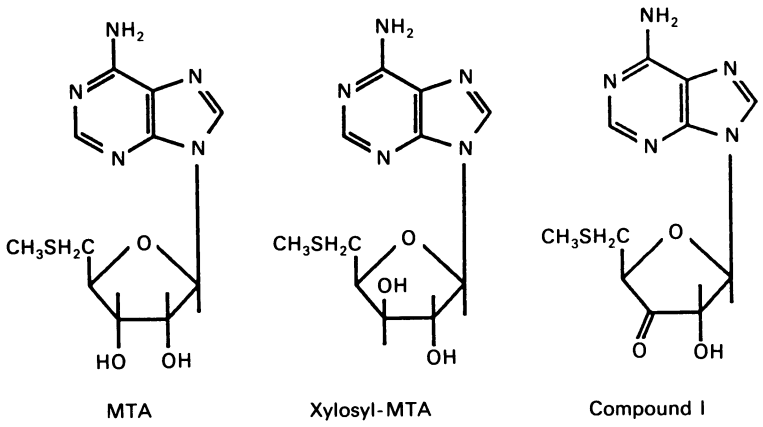


Fig. 1. Structural formulae of 9-[5′-deoxy-5′-(methylthio)-β-D-ribofuranosyl]adenine (MTA), 9-[5′-deoxy-5′-(methylthio)-β-D-xylofuranosyl]adenine (xylosyl-MTA) and 9-[5′-deoxy-5′-(methylthio)-β-D-*erythro*-pentofuran-3′-ulosyl]adenine (compound I)

ion-exchange chromatography (Shapiro & Ehninger, 1966). MTA and [*Me*-^{14}C]MTA were prepared by acid hydrolysis (pH 4.5 at 100 °C for 30 min) of AdoMet and [*Me*-^{14}C]AdoMet respectively (Schlenk & Ehninger, 1964). Xylosyl-MTA was isolated and purified from *D. verrucosa* (Cimino *et al.*, 1986). Putrescine, spermidine, spermine and 1,5-diaminopentane were from Janssen Chimica (Brussels, Belgium). [8-^{14}C]Adenine, NaB^3H_4, [1,4-^{14}C]putrescine, [1,4-^{14}C]spermidine and [*Me*-^{14}C]-AdoMet were supplied by Amersham International (Amersham, Bucks., U.K.). *S*-Adenosyl(5′)-3-methylthio[*Me*-^{14}C]propylamine was obtained from [*Me*-^{14}C]AdoMet by enzymic decarboxylation (Wickner *et al.*, 1970). The chemical purity of the standards was checked by paper chromatography, t.l.c. and h.p.l.c. (Zappia *et al.*, 1980*b*). Other chemicals, of analytical-reagent grade, were obtained from conventional commercial sources.

Enzymes

MTA phosphorylase (EC 2.4.2.1) was partially purified from *D. verrucosa* through an $(NH_4)_2SO_4$ fractionation step as previously described (Cacciapuoti *et al.*, 1978).

Spermidine synthase and spermine synthase activities were assayed as described by Raina *et al.* (1983) by monitoring the formation of [*Me*-^{14}C]MTA from *S*-adenosyl(5′)-3-methylthio[*Me*-^{14}C]propylamine in the presence of the appropriate polyamine acceptor.

H.p.l.c. analysis

A Beckman (Fullerton, CA, U.S.A.) model 324 liquid chromatograph equipped with a model 100-40 spectrophotometric detector was used. Reverse-phase h.p.l.c. analyses were performed on a column (25 cm × 4.6 mm internal diam.) of Ultrasil ODS RP-18 (Beckman). The elution was carried out with methanol/20 mM-ammonium formate, pH 4 (3:7, v/v), at a flow rate of 1 ml/min. Ion-exchange h.p.l.c. analyses were performed on a column (25 cm × 4.6 mm internal diam.) of Ultrasil SCX (Beckman). The elution was carried out with 0.5 M-ammonium formate, pH 4, at a flow rate of 1 ml/min. Injection was performed with a model 7120 sample-injector valve (Rheodyne; Cotati, IL, U.S.A.).

Experiments performed *in vivo*

In each incorporation experiment, specimens of *D. verrucosa* were placed in aerated seawater, and the appropriate labelled precursor dissolved in saline (0.9% NaCl) was injected into the hepatopancreas of each animal by means of a 1 ml glass syringe; 50 μl or 100 μl portions were injected into each animal. This treatment apparently has no significant noxious effect on the mollusc. At different time intervals the animals were killed and homogenized in ice-cold 1.5 M-$HClO_4$ (1:4, w/v). After centrifugation, the deproteinized supernatant was neutralized with KOH and centrifuged. Assay of MTA and xylosyl-MTA was performed by reverse-phase h.p.l.c., and AdoMet was detected by ion-exchange h.p.l.c. Determination of radioactivity in the h.p.l.c. eluate was performed in a 1 ml sample added to 4 ml of Insta-Gel (Packard).

The precipitate obtained from homogenate after deproteinization with $HClO_4$ was washed twice by suspension in 2 ml of water followed by centrifugation. Portions (0.1 g) of the final precipitate were dissolved by digestion with 2 ml of Solulyte (Baker)/propan-2-ol (1:1, v/v) at 50 °C for 24 h in a shaking water bath. The samples were then bleached with a few drops of H_2O_2. The scintillation fluid was Dynagel (Baker)/HCl (10:1, v/v). Absolute radioactivity was measured in a Beckman LS 7800 scintillation counter, equipped with an automatic quench correction system (AQC).

Determination of AdoMet, MTA, xylosyl-MTA and polyamines in *D. verrucosa*

AdoMet was determined by the method of Zappia *et al.* (1983). Neutralized $HClO_4$ extracts were analysed by h.p.l.c. as described previously (Zappia *et al.*, 1983; Porcelli *et al.*, 1988*a*).

MTA was determined by h.p.l.c. analysis as described by Della Ragione *et al.* (1981).

Xylosyl-MTA was determined as previously reported (Porcelli *et al.*, 1988*b*) by reverse-phase h.p.l.c. by comparison of absorbance integrated peak areas with calibration graphs obtained with pure xylosyl-MTA.

Quantitative determination of polyamines was carried out by reverse-phase h.p.l.c. after reaction with benzoyl chloride as described by Redmond & Tseng (1979).

Synthesis of [3'-^{3}H]MTA and [3'-^{3}H]xylosyl-MTA

A 42 mg portion of compound II, prepared as previously reported (Gavagnin & Sodano, 1989), was dissolved in 1.5 ml of ethanol. The solution was cooled at 0 °C (ice bath) and 10 mCi of NaB^3H_4 (196 mCi/mmol) was added under stirring. After 2 h methanol was added. After evaporation of the solvents the material was partitioned between saturated aq. NaCl and ethyl acetate. The organic phase was carried over Na_2SO_4, concentrated to a small volume and applied to two semi-preparative silica-gel t.l.c. plates, which were eluted with ethyl acetate. A u.v.-absorbing band (R_F 0.3) was scraped from the plate and eluted with ethyl acetate, affording a mixture of 2'-*O*-(t-butyldimethylsilyl) derivatives of [3'-^{3}H]MTA and [3'-^{3}H]xylosyl-MTA (17 mg; 2.4×10^8 d.p.m./mg). The above mixture was dissolved in tetrahydrofuran (2 ml); 100 μl of 1 M-tetra-n-butylammonium fluoride in tetrahydrofuran was added and the solution was stirred for 30 min at room temperature. After evaporation of solvent, the material was purified on two semi-preparative silica-gel t.l.c. plates with chloroform/methanol (4:1, v/v) as solvent. Two u.v. absorbing bands were eluted with chloroform/methanol (4:1, v/v), giving [3'-^{3}H]xylosyl-MTA (5 mg; 3.2×10^8 d.p.m./mg) and [3'-^{3}H]MTA (3 mg; 3.5×10^8 d.p.m./mg). The identity of the two compounds was confirmed by t.l.c. and h.p.l.c. comparison with authentic non-labelled samples.

The compounds [3'-^{3}H]MTA and [3'-^{3}H]xylosyl-MTA were further purified, before the experiments performed *in vivo*, by reverse-phase h.p.l.c. on an Ultrasil ODS RP-18 column (Beckman).

Ichthyotoxicity test

Ichthyotoxicity assay was conducted with the mosquito fish, *Gambusia affinis* (Baird and Girard), by using the procedure described by Gunthorpe & Cameron (1987) and Coll *et al.* (1982). Six fish were placed in distilled water (70 ml) and an acetone solution (0.5 ml) of xylosyl-MTA (0.7 mg) was added. No toxic effects were observed within 24 h.

Protein determination

Protein concentration was determined by the method of Bradford (1976), with human immunoglobulin as a standard.

RESULTS AND DISCUSSION

The concentrations of AdoMet, MTA, xylosyl-MTA and polyamines in *D. verrucosa* were measured and the results are reported in Table 1. The measurements were made on homogenates of the whole organism. The cellular concentration of AdoMet ranges from 30 to 35 nmol/g, of the same order of magnitude as that reported in the literature for eukaryotic tissues (Hibasami *et al.*, 1980). In order to check the occurrence of a xylosyl analogue of AdoMet as a possible precursor of xylosyl-MTA, the pool of the sulphonium compounds, isolated and purified from *D. verrucosa* homogenates by the standard chromatographic procedures (Zappia *et al.*, 1980*b*), was subjected to acid hydrolysis at pH 4.5 at 100 °C for 1 h, conditions in which AdoMet and its putative analogue are quantitatively cleaved into MTA and xylosyl-MTA respectively (Schlenk & Ehninger, 1964). Since reverse-phase h.p.l.c. analysis of the hydrolysis mixture did not reveal the presence of xylosyl-MTA, it is possible to exclude the occurrence of a sulphonium compound modified in the sugar moiety.

Table 1. MTA, xylosyl-MTA, AdoMet and polyamine concentrations in *D. verrucosa*

Compound	Cellular concentration (nmol/g of wet tissue)
MTA	2±0.3
Xylosyl-MTA	355±25
AdoMet	32±3
Putrescine	64±3
Cadaverine	5±0.3
Spermidine	78±4
Spermine	56±3

The concentration of MTA in *D. verrucosa* is 2± 0.3 nmol/g, similar to that reported in the literature for eukaryotic tissues (Seidenfeld *et al.*, 1980; Della Ragione *et al.*, 1981), indicating that the thioether is actively metabolized in the mollusc. In fact MTA phosphorylase, the enzyme devoted to MTA catabolism, was found to be present in *D. verrucosa* tissues with a specific activity of 0.1 nmol/min per mg of protein, which is slightly lower than that of other animal tissues (Zappia *et al.*, 1983; Della Ragione *et al.*, 1986).

The concentrations of polyamines, reported in Table 1, are of the same order of magnitude as those reported for eukaryotic tissues (Bachrach *et al.*, 1982) and indicate that the biosynthesis of these polycations is operative in *D. verrucosa*.

As recently reported (Porcelli *et al.*, 1988*b*), xylosyl-MTA is totally resistant to enzymic cleavage by MTA phosphorylase. This result is consistent with the elevated concentration of the molecule found in the mollusc (330–380 nmol/g).

In order to evaluate the effect of xylosyl-MTA on the enzymes involved in MTA metabolism, it was assayed as a potential inhibitor of MTA phosphorylase, spermidine synthase and spermine synthase partially purified from *D. verrucosa*. Assayed at concentrations up to 500 μM, xylosyl-MTA did not display any inhibitory effect on these enzymic systems (results not shown), indicating that this molecule does not interfere with MTA catabolism or with its regulatory effects on polyamine biosynthesis. Probably the chirality of C-3' of the ribosyl moiety of MTA is critical in the process of recognition by spermidine synthase, spermine synthase and MTA phosphorylase.

Experiments performed *in vivo* with as precursors [*Me*-^{14}C]methionine, [*Me*-^{14}C]MTA and [8-^{14}C]adenine were carried out to study the biogenesis of xylosyl-MTA. The molecules were injected directly into the digestive gland of *D. verrucosa*. Treated animals were killed 1 h, 2 h and 24 h after the injection. Each experiment was carried out by treating two animals and pooling together the homogenates, which were then subjected to the procedure described in the Materials and methods section. The results of these experiments are summarized in Table 2. After 1 h, in the animals treated with labelled methionine, most of radioactivity recovered in the supernatant is associated with AdoMet and only 2.3% is detectable as xylosyl-MTA. After 2 h xylosyl-MTA accounts for 28% of the radioactivity but MTA is barely detectable. This

Table 2. Incorporation of labelled precursors *in vivo* in *D. verrucosa*

Into each animal was injected 50 μl of a saline solution containing [8-^{14}C]adenine (5×10^6 d.p.m.; 54 mCi/mmol), [*Me*-^{14}C]methionine (5×10^6 d.p.m.; 58 mCi/mmol) or [*Me*-^{14}C]MTA (1.2×10^6 d.p.m.; 55 mCi/mmol). Abbreviation: N.D., not detected.

Precursor	Time after injection (h)	No. of animals	Radioactivity recovered (d.p.m.)		Radioactivity incorporated (% of radioactivity recovered in supernatant)		
			Supernatant	Precipitate	AdoMet	MTA	Xylosyl-MTA
[8-^{14}C]Adenine	1	2	2900000	500000	0.1	N.D.	2.34
	2	2	2600000	800000	N.D.	N.D.	2.42
[*Me*-^{14}C]Methionine	1	2	3000000	1200000	8.3	N.D.	2.3
	2	2	1400000	1700000	12.14	0.57	28.0
[*Me*-^{14}C]MTA	1	2	600000	250000	N.D.	4.0	33.0
	2	2	670000	300000	N.D.	4.4	62.6
	24	2	500000	480000	N.D.	N.D.	72.0

result is in agreement with the high concentrations of xylosyl-MTA found in *D. verrucosa*, suggesting that the xylosyl thioether can be envisaged as an accumulation product. It may be noted that 65% of the administered radioactivity is still retained in the animals 2 h after the injection.

When the animals were injected with labelled adenine, no loss of radioactivity was observed, since most of the administered radioactivity is still present in the animals even 2 h after the treatment. However, because of the multiple biosynthetic pathways involving adenine, no detectable radioactivity appears to be associated with AdoMet and MTA, molecules characterized by a high turnover rate. On the other hand, xylosyl-MTA accounts for 2.4% of the radioactivity recovered in the supernatant after 2 h. From this experimental approach, it is possible to infer that the xylosyl analogue derives from methionine and adenine through the usual biosynthetic route in which AdoMet represents the intermediate.

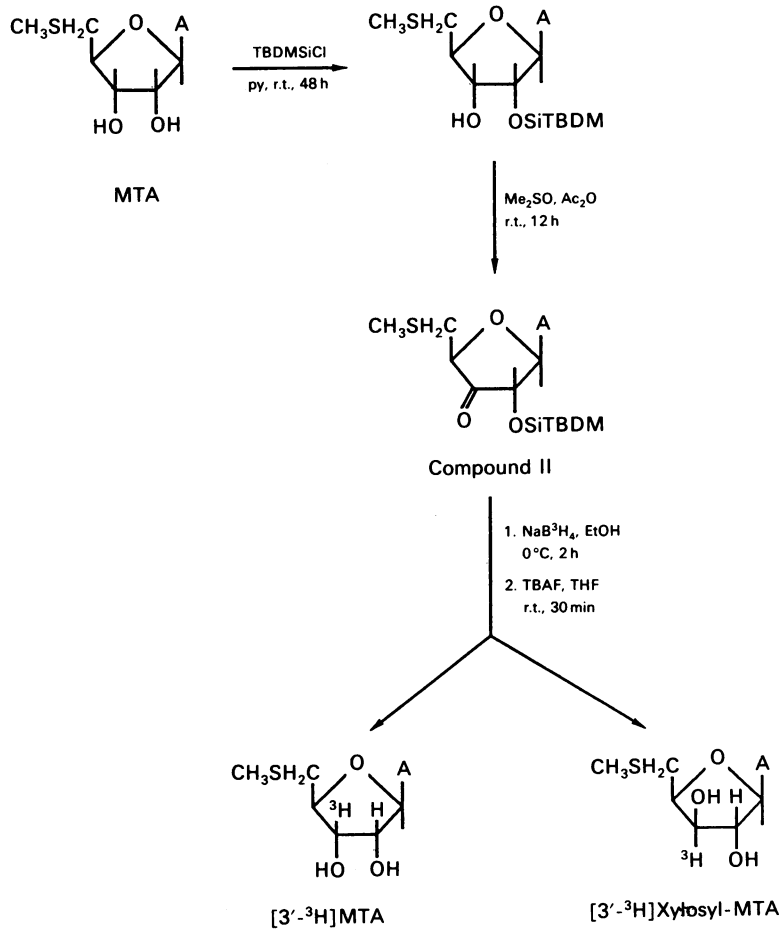
A better understanding of the biogenetic origin of xylosyl-MTA comes from the analysis of the third experiment. When the animals were injected with [*Me*-^{14}C]MTA, no radioactivity was associated with AdoMet, nor was excretion of labelled compounds observed. It is worth noting that MTA is rapidly metabolized in that only 4% of the radioactivity recovered in the supernatant is associated with this molecule after 1 h. On the other hand, the high level of radioactivity incorporated into xylosyl-MTA (33.0%) suggests that MTA is a close precursor of the analogue. Despite the low concentration of radiolabelled MTA present at 1 h and 2 h, xylosyl-MTA increases significantly after 2 h and after 24 h; thus it is possible to hypothesize that the conversion of MTA into xylosy-MTA occurs through an intermediate(s) that is quickly formed from MTA and gives rise to xylosyl-MTA more slowly.

In order to ascertain whether an oxidation–reduction process at C-3′ is involved in the MTA–xylosyl-MTA conversion, the fate of the 3′-proton of MTA was investigated by ^{3}H-labelling.

The synthesis of [3′-^{3}H]MTA was carried out as reported in Scheme 1. It has recently been shown (Gavagnin & Sodano, 1989) that $NaBH_4$ reduction of the ketone compound II proceeds in a non-stereospecific fashion, affording, after deprotection, an approximately 2:1 mixture of the analogue (xylosyl-MTA) and MTA. NaB^3H_4 reduction of compound II proceeds similarly, yielding the 3′-tritiated compounds xylosyl-MTA and MTA, which were separated by preparative silica-gel t.l.c. and further purified by reverse-phase h.p.l.c.

[3′-^{3}H]MTA was mixed with [*Me*-^{14}C]MTA to a 3.1:1 ^{3}H/^{14}C ratio, and portions of this mixture were injected into six specimens of *D. verrucosa*. The animals were killed two at a time after 1 h, 2 h and 24 h intervals and treated as described above. Measurements of the radioactivity of the metabolites after h.p.l.c. separation showed (Table 3) that the recovered xylosyl-MTA was labelled to a considerable extent and retained only ^{14}C radioactivity, the ^{3}H label of the precursor having been lost during the MTA–xylosyl-MTA conversion. This evidence strongly favours an intermediary presence of a 3′-oxo derivative of MTA during its conversion into xylosyl-MTA. On the other hand, the MTA recovered from these experiments showed a ^{3}H/^{14}C ratio (Table 3) decreasing from 2.67:1 after 1 h to 0.46:1 after 24 h. A possible explanation for this behaviour could be that the reduction of the hypothetic ketone intermediate *in vivo* proceeds with low stereospecificity, giving rise to both xylosyl-MTA and MTA. This latter is transformed into the former by an iterative process since 60.4% of the ^{14}C radioactivity in the supernatant after 24 h was found to be associated with xylosyl-MTA and only 6.2% with MTA. In addition, the possibility of a loss of ^{3}H at C-3 during the resynthesis of MTA from 5-methylthioribose 1-phosphate could also be taken into account.

The [3′-^{3}H]xylosyl-MTA obtained from the above synthesis was injected into six specimens of *D. verrucosa* to investigate the turnover rate of this compound. The very high proportion of the recovered radioactivity associated with xylosyl-MTA, almost invariable during the time span (Table 4), suggests that the xylosyl-MTA has a very low turnover rate and therefore accumulates in the mollusc. Accordingly, this finding precludes the possibility that the xylosyl-MTA could be transformed back into MTA to an appreciable extent, as also indicated by the small amounts of radioactivity found in the precipitate resulting from cleavage of MTA and incorporation of radioactivity into proteins (compare the radioactivity values of the precipitates when labelled MTA was administered).



Scheme 1. Procedure for the synthesis of [3′-^{3}H]MTA and [3′-^{3}H]xylosyl-MTA

Abbreviations: TBDMSiCl, t-butyldimethylsilyl chloride; py, pyridine; Me_2SO, dimethyl sulphoxide; Ac_2O, acetic anhydride; EtOH, ethanol; TBAF, tetra-n-butylammonium fluoride; THF, tetrahydrofuran; r.t., room temperature.

Table 3. Incorporation experiments with [3′-^{3}H,*Me*-^{14}C]MTA in *D. verrucosa*

A total of 15×10^6 d.p.m. of [3′-^{3}H]MTA (45 mCi/mmol) and of 4.8×10^6 d.p.m. of [*Me*-^{14}C]MTA (55 mCi/mmol) was injected in the six animals (^{3}H/^{14}C ratio 3.1:1). A 600 μl saline solution containing the mixture of the two labelled precursors was taken up with a 1 ml glass syringe and approx. 100 μl was injected into each animal.

		Radioactivity recovered (d.p.m.)						Radioactivity incorporated (% of radioactivity recovered in supernatant)				
		Supernatant			Precipitate			MTA			Xylosyl-MTA	
Time after injection (h)	No. of animals	^{3}H	^{14}C	^{3}H/^{14}C ratio	^{3}H	^{14}C	^{3}H/^{14}C ratio	^{3}H	^{14}C	^{3}H/^{14}C ratio	^{3}H	^{14}C
1	2	1788000	884000	2.02:1	82000	119000	0.69:1	10.8	8.3	2.67:1	–	23.3
2	2	1574000	1091000	1.44:1	83000	151000	0.55:1	5.2	9.8	0.76:1	–	47.0
24	2	667000	912000	0.73:1	53000	340000	0.15:1	3.8	6.2	0.46:1	–	60.4

CONCLUSION

The results provide strong evidence for the hypothesis that xylosyl-MTA arises in *D. verrucosa* from isomerization of endogenous MTA. The overall pathway formally consists in an inversion of configuration at C-3′ of MTA and should involve oxidation followed by reduction, since the H-3′ is lost during the process. Presumably a stable intermediate is formed during isomerization; this intermediate could be envisaged as

Table 4. Recovery of radioactivity in *D. verrucosa* after injection of [3′-^{3}H]xylosyl-MTA

A total of 15×10^6 d.p.m. of [3′-^{3}H]xylosyl-MTA (43.5 mCi/mmol) was injected into the six animals. A 600 μl saline solution containing the labelled precursor was taken up with a glass syringe and approx. 100 μl was injected into each animal.

Time after injection (h)	No. of animals	Radioactivity recovered (d.p.m.)		Radioactivity incorporated into xylosyl-MTA (% of radioactivity recovered in supernatant)
		Supernatant	Precipitate	
1	2	3528000	85000	86.4
2	2	2659000	43000	85.3
24	2	5661000	94000	96.0

the ketone compound I (Fig. 1) or a close derivative. Enzymes involved in the MTA–xylosyl-MTA transformation should include an epimerase acting via an oxidation–reduction mechanism analogous to that reported in the literature for other substrates (Glaser, 1972).

Since xylosyl-MTA is resistant to enzymic cleavage by MTA phosphorylase and does not isomerize back into MTA, it accumulates in the mollusc. Furthermore, xylosyl-MTA does not inhibit MTA phosphorylase itself, nor does it inhibit spermidine synthase and spermine synthase, and therefore it does not interfere with MTA catabolism and its regulatory effects on polyamine biosynthesis. From these data xylosyl-MTA should be regarded as a typical secondary metabolite that apparently does not play a relevant role in the primary metabolism of *D. verrucosa*. Moreover, since xylosyl-MTA is not toxic to fish, it is presumably not involved in the defence mechanism of the nubidranch (Karuso, 1987). Interestingly, ichthyotoxic diacylglycerols, which do account for the chemical defence, have been isolated from the same animal (Cimino *et al.*, 1988).

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REFERENCES

Bachrach, U., Kaye, A. & Chayen, R. (eds.) (1982) Adv. Polyamine Res. **4**

Bradford, M. M. (1976) Anal. Biochem. **72**, 248–254

Cacciapuoti, G., Oliva, A. & Zappia, V. (1978) Int. J. Biochem. **9**, 35–41

Cimino, G., Crispino, A., De Stefano, S., Gavagnin, M. & Sodano, G. (1986) Experientia **42**, 1301–1302

Cimino, G., Gavagnin, M., Sodano, G., Puliti, R., Mattia, C. A. & Mazzarella, L. (1988) Tetrahedron **44**, 2301–2310

Coll, J. C., La Barre, S., Sammarco, P. W., Williams, W. T. & Bakus, G. J. (1982) Mar. Ecol. Prog. Ser. **8**, 271–278

Della Ragione, F., Cartenì-Farina, M., Porcelli, M., Cacciapuoti, G. & Zappia, V. (1981) J. Chromatogr. **226**, 243–249

Della Ragione, F., Cartenì-Farina, M., Gragnianiello, V., Schettino, M. I. & Zappia, V. (1986) J. Biol. Chem. **261**, 12324–12329

Della Ragione, F., Cartenì-Farina, M. & Zappia, V. (1989) in The Physiology of Polyamines (Bachrach, U. & Heimer, Y. M., eds.), vol. 1, pp. 231–251, CRC Press, Boca Raton

Di Fiore, P. P., Grieco, M., Pinto, A., Attadia, V., Porcelli, M., Cacciapuoti, G. & Cartenì-Farina, M. (1984) Cancer Res. **44**, 4096–4103

Gavagnin, M. & Sodano, G. (1989) Nucleosides Nucleotides, **8**, in the press

Glaser, L. (1972) Enzymes 3rd Ed. **6**, 355–380

Gunthorpe, L. & Cameron, A. M. (1987) Mar. Biol. **94**, 39–43

Hibasami, H., Hoffman, J. L. & Pegg, A. E. (1980) J. Biol. Chem. **255**, 6675–6678

Karuso, P. (1987) in Bioorganic Marine Chemistry (Scheuer, P. J., ed.), pp. 31–60, Springer-Verlag, New York

Pajula, R. L. & Raina, A. (1979) FEBS Lett. **99**, 343–347

Pegg, A. E. & Williams-Ashman, H. G. (1986) in Inhibition of Polyamine Metabolism: Biological Significance and Basis for New Therapies (McCann, P. P., Pegg, A. E. & Sjoerdsma, A., eds.), pp. 33–48, Academic Press, New York

Pegg, A. E., Borchardt, R. T. & Coward, J. K. (1981) Biochem. J. **194**, 79–89

Porcelli, M., Cacciapuoti, G., Gambacorta, A. & Cartenì-Farina, M. (1988*a*) Eur. J. Biochem. **177**, 273–280

Porcelli, M., Cacciapuoti, G., Oliva, A. & Zappia, V. (1988*b*) J.Chromatogr. **440**, 151–155

Porcelli, M., Cacciapuoti, G. Cimino, G., Gavagnin, M., Sodano, G. & Zappia, V. (1989) in Progress in Polyamine Research: Novel Biochemical, Pharmacological and Clinical Aspects (Zappia, V. & Pegg, A. E., eds.), pp. 219–228, Plenum Press, New York

Raina, A., Eloranta, T. & Pajula, R. L. (1983) Methods Enzymol. **94**, 257–260

Redmond, J. W. & Tseng, A. (1979) J. Chromatogr. **170**, 479–481

Schlenk, F. & De Palma, R. E. (1957) J. Biol. Chem. **229**, 1037–1050

Schlenk, F. & Ehninger, D. J. (1964) Arch. Biochem. Biophys. **106**, 95–100

Seidenfeld, J., Wilson, J. & Williams-Ashman, H. G. (1980) Biochem. Biophys. Res. Commun. **95**, 1961–1967

Shapiro, S. K. & Ehninger, D. J. (1966) Anal. Biochem. **15**, 323–333

Wickner, R. B., Tabor, C. W. & Tabor, H. (1970) J. Biol. Chem. **245**, 2132–2139

Williams-Ashman, H. G., Seidenfeld, J. & Galletti, P. (1982) Biochem. Pharmacol. **31**, 277–288

Zappia, V., Cartenì-Farina, M., Cacciapuoti, G., Oliva, A. & Gambacorta, A. (1980*a*) in Natural Sulfur Compounds: Novel Biochemical and Structural Aspects (Cavallini, D., Gaull G. E. & Zappia, V., eds.), pp. 133–148, Plenum Press, New York

Zappia, V., Galletti, P., Porcelli, M., Manna, C. & Della Ragione, F. (1980*b*) J. Chromatogr. **189**, 399–405

Zappia, V., Cartenì-Farina, M., Galletti, P., Della Ragione, F. & Cacciapuoti, G. (1983) Methods Enzymol. **94**, 57–66

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