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Biomethylation of tin (II) complexes in the presence of pure strains of *Saccharomyces cerevisiae*

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The methylation of several tin compounds by strains of *Saccharomyces cerevisiae* strains (yeast) is described. The production of the methyl tin species was established by gas chromatography and mass spectrometry. Monomethyl tin (IV) products dominated but some dimethyl tin products were observed. This appears to be the first report of a tin methylation by a pure strain of a methylating species rather than a complex mixture obtained from an environmental source.

Keywords: Biomethylation, methyl tin compounds, environmental methylation, *Saccharomyces cerevisiae*, yeasts

INTRODUCTION

It is by now well known that exposure of inorganic mercury compounds to natural waters, microorganisms or sediments may lead to the production of methyl mercury species (biomethylation).[1] In addition to the intrinsic interest of the mechanistic steps in the environmental addition of methyl groups to metals, it should also be realized that the methyl metal derivatives so produced are usually more toxic than the inorganic metal substrates from which they derive (arsenic is the exception here). The biochemistry of metal methylation in the environment is not clearly understood. Methyl cobalamin derivatives (the methyl analogue of vitamin B_{12}, CH_3B_{12}, where the methyl group is directly attached to cobalt) have been identified as the proximate methyl source in several instances,[2] but other methyl sources must also exist.[3] Several other metals or metalloids have been found in their methyl forms in the natural environment (e.g. chiefly, tin, lead, germanium, arsenic, antimony, selenium)[4] and numerous authors have suggested that they are formed there. In the case of lead, this suggestion is controversial owing to the widespread use of methyl lead compounds in their environmentally dispersive role as petrol additives.

There are by now numerous reports of the discovery of methyl tin species in the aqueous natural environment.[5–9] It is usually assumed that they are formed there as there are few methyl tin products that could have dispersed to account for their environmental existence. (Obviously this argument does not apply to butyl tins found in the aquatic environment as these will have arisen from the use of butyl tin-containing products.) However there are some limited uses of methyl tins in several products, e.g. as stabilizers in PVC water pipes,[10] and the possibility of methyl tin impurities in other organo-tin products also exists. The discovery of methyl tin compounds in the environment is strong circumstantial evidence for biomethylation; but it is not conclusive. To date low concentrations of $(CH_3)_nSn^{(4-n)+}$ species (at approximately the ng dm^{-3} level) have been analysed from harbours, estuaries, rivers and ocean waters.[5–9] Unexpectedly, methyl tin hydrides $[(CH_3)_nSnH_{4-n}]$ have also been detected in anoxic harbour muds.[7] In an aerobic environment they would be expected to have little stability even in attentuated form.[11] There have been reports of methyl butyl tin compounds in harbour sediments[12] where the methyl group is implicitly assumed to have been added to a tributyl or dibutyl moiety arising from an anti-fouling paint i.e. biomethylation of tributyl tin. However it is not impossible that the methyl butyl tin species was already present as an impurity in the original paint. Abiotic and biotic pathways for the methylation of trimethyl tin compounds have been proposed.[13]

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There have to date been only two reports of an incubation of an inorganic tin compound with a biological medium to produce a methyl tin product.[7,14] In one experiment tin (IV) chloride ($SnCl_4 5H_2O$) was converted to tri- and dimethyl tin species by incubation with aliquots of natural sediments from Chesapeake Bay, USA. The source of the methyl groups was identified as sediment microflora but no individual identifications could be made. Only small traces of monomethyl tin species were found in this work. In the other report inorganic tin (II) and tin (IV) compounds were incubated with an aerobic strain of Pseudomonas 244 (Ps 244) also isolated from Chesapeake Bay. Tin (IV) species gave various methyl stannanes [$(CH_3)_4SnH_{4-n}, n=2,3,4$] after hydride generation analysis but tin (II) was reported to produce only trace amounts of methyl tin products.[7]

In the present work we report the reproducible production of methyl tin species from the incubation of several inorganic and metal-organic tin (II) complexes with yeast and other fungal strains. In our work the dominant product, monomethyl tin, is the expected initial compound produced by a single step oxidative carbonium ion (CH_3^+) transfer to tin (II) to produce tin (IV) products.[15,16] Small traces of dimethyl tin are sometimes seen and may arise either from further methylation by a different non-oxidative mechanism or by dismutation of the initial monomethyl product.[17]

EXPERIMENTAL

The experiments were carried out as follows:

Flasks containing 50 cm^3 of malt extract broth, together with approximately 0.1 g (accurate measure) of tin compound were sterilised by autoclaving at 130°C. After cooling, approximately 1 g of the fungal species was added using aseptic technique and the flasks kept at room temperature in the dark. 10 cm^3 samples of the liquid phase were withdrawn at appropriate intervals for analysis of any methyl tin products by a hydride generation technique[18] after filtration to separate the liquid phase from the solid yeast cell debris. The tin compounds used were prepared by literature methods.[19]

The methyltin products were analysed by gas chromatography (GC) after generation of the hydrides with sodium borohydride. 10 cm^3 of reaction solution in 50 cm^3 vials was buffered with 2 mol dm^{-3} tris-HCL pH 6.5 buffer and the vials sealed with teflon lined crimp-on caps. The hydrides were generated by injecting 2 cm^3 of 1.5% w/v sodium borohydride solution and 1 cm^3 of headspace used for analysis. Trimethyl tin chloride was used as internal standard for some experiments. 1 cm^3 samples of headspace products could be injected reproducibly.

A Pye 104 gas chromatograph with flame ionisation detector, fitted with a glass column 1800 mm in length by 0.4 mm i.d. was used. The column was packed with 10% SP2100 on

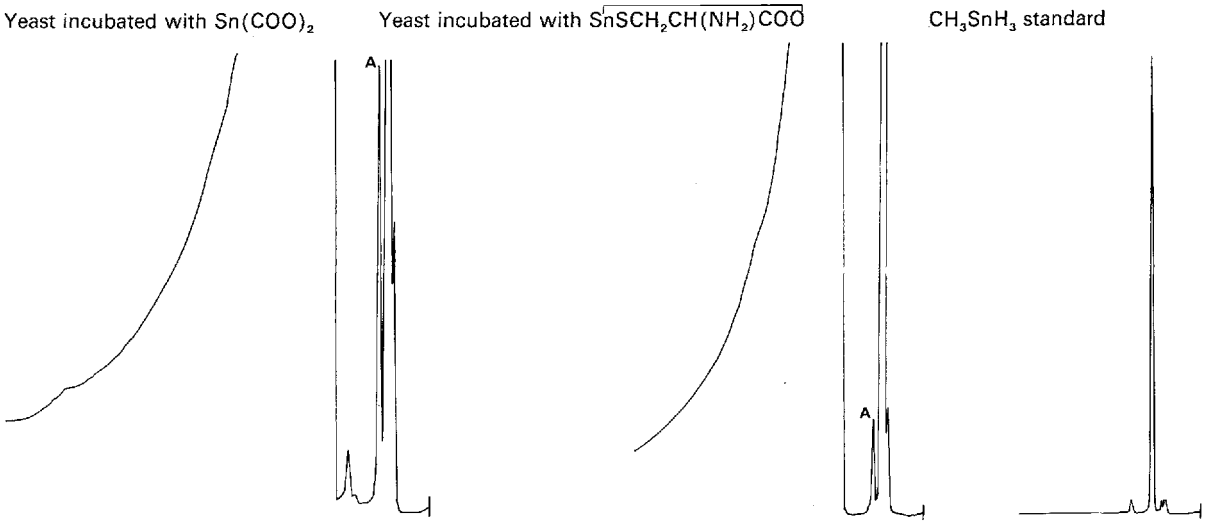

Figure 1 GC traces of the incubation of tin complexes with *Saccharomyces cerevisiae* (yeast). Peaks 'A' are CH_3SnH_3, retention time 2 min. Blank traces of yeast only and tin complex only showed no peak at this retention time.

chromosorb W80/100 mesh; oven, detector and injector temperatures were 30°C, 40°C and 30°C respectively. Carrier gas was oxygen-free nitrogen, flow rate 20 $cm^3 min^{-1}$. Under these conditions the retention time for methyl tin trihydride was 2 minutes.

A magnetic deflection micromass 16-F instrument was used for GC mass spectroscopy (GCMS) coupled to a Pye 204 GC system fitted with a 1% SP2100 50m capillary or other column (WCOT) with helium carrier gas flow rate 2 $cm^3 min^{-1}$. Specific m/e values were

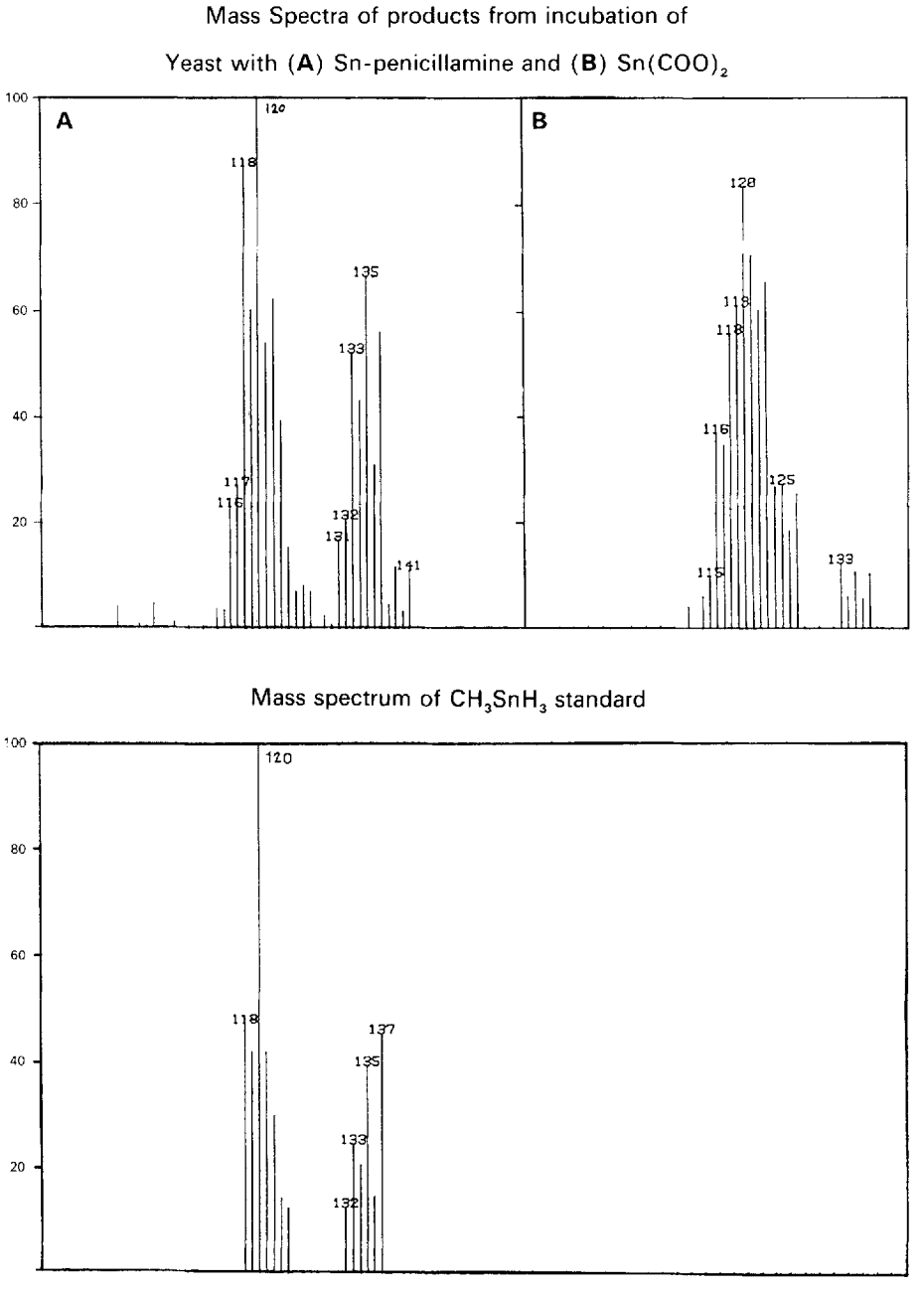

Figure 2 Ms patterns of the products from incubation of tin complexes with *Saccharomyces cerevisiae* (yeast). Also MS of a standard sample of CH_3SnH_3 (hydride generated).

located by Multiple Ion monitoring using a VG Digital Scan Generator, allowing a 100-fold improvement in detection over samples without DSG.

Following growth of the yeast strain in the presence of a range of tin compounds including Sn, Sn(II) and Sn(IV) species, methyl tin products were observed only for tin (II) complexes of sulphur containing amino-acids and for tin (II) oxalate. Amounts of methyl tin produced from the amino-acid complexes ranged from 48.3 μg for tin (II) penicillamine to 3.45 μg for tin (II) methionine (Table 1). Tin (II) oxalate produced 11.8 μg.

Table 1 Methylation of tin species from incubation with *Saccharomyces cerevisiae* (yeast)

Tin compound	Yield (%)	Amount of CH_3Sn produced[a]
Sn°	—	—
SnS	<0.002	<1
$SnCl_4$	—	—
$\overline{SnSC(CH_3)_2CHNH_2COO}$	0.05	48.3
$\overline{SnSCH_2CHNH_2COO}$	0.01	9.7
$Sn[CH_3S(CH_2)_2CHNH_2COO]_2$	0.006	3.45
$Sn(COO)_2$	0.012	11.6

Conditions
Room Temperature; aerobic; Standard media; Dark: Time—2 days; 100 mg tin species
Amount of *Saccharomyces cerevisiae*—1 g in 50 cm^3 media

[a]As μg CH_3SnH_3(2 days).

The production of monomethyl tin (IV) was also followed over a time period for the yeast-tin (II) oxalate system and a plot of dry weight of yeast versus monomethyl tin production was obtained. The amount of monomethyl tin (expressed as μg of CH_3SnH_3) present in the culture media +cells increased during the rapid growth phase of the yeast, reaching a maximum as the dried weight of the cells reached a maximum, thereafter declining rapidly.

Tin (II) chloride was not shown to be methylated but this may be due to the instability of tin (II) chloride to oxidation under these conditions. Any methyl tin produced here may therefore be below the present detection limit of 0.5 μg of CH_3SnH_3 for this system.

RESULTS AND DISCUSSION

The successful methylation of the tin amino-acid and oxalate complexes may be due in part to increased resistance of the Sn(II) to oxidation in solution and hence the availability of electron rich tin (II) centres for a carbonium ion methylation process as described by Challenger for Arsenic (III).[20]

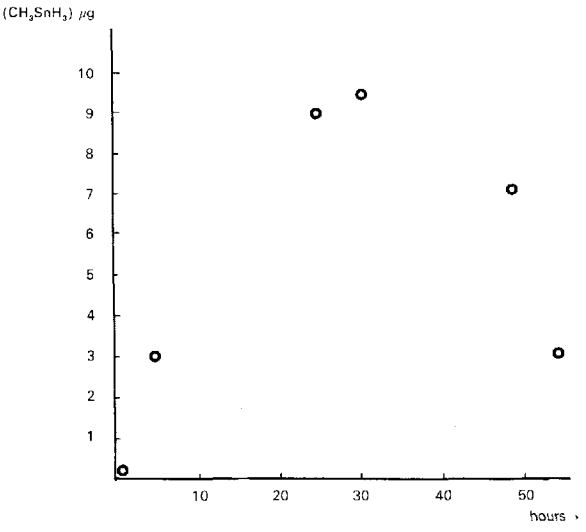

Figure 3 Production of CH_3SnH_3 over a time period from the incubation of tin (II) oxalate with *Saccharomyces cerevisiae*. CH_3SnH_3 is total methyl tin in medium and yeast cells in μg.

Three strains of brewers yeasts were also incubated in the presence of the tin (II) compounds, giving similar results. It seems likely therefore that the ability of *Saccharomyces cerevisiae* to methylate tin may be a general property of this species.

Methylation of these tin (II) compounds with yeast strains has suggested further work with other fungi potentially capable of methylation of Group IV metals by carbonium ion transfer. These results will be described in a future communication.

The yields over the period of the experiment, as based on tin, are small, but several points should be made here. First most of the tin compounds had almost no effective solubility in the aqueous medium and hence accessible tin concentrations may have been much (perhaps up to about 10^3) lower than added tin concentrations. This raises yields stated correspondingly. In addition only a single batch of nutrient was added and the yeast growth was in a dormant stage at the conclusion of the experiments. Hence the stated yields only reflect production after two days of active growth. In our experiments there

was no visible growth of yeast beyond about 2 days and no apparent production of methyl tin either. We are currently experimenting with a continous culture method to produce yeast growth in the presence of tin over a much longer period. Methyl tin yields from such experiments are likely to be proportionately larger than over the 3 day experiments.

Care has been taken to ensure that the methyl tin species observed are not experimental artefacts. Identification is by GC-flame ionisation detection, supported in each case by the absolute technique of GC-MS (Fig. 2). Sterile tin-only blanks, tin plus sterile yeast blanks, active and sterile yeast blanks all produced no trace of methyl tin products on hydride generation. Some experimental tin (II) compounds (e.g. $SnCl_2$) produced no methyl tins on being subjected to the full experimental procedure. The reliability and sensitivity of the analytical technique is reported elsewhere and is well within the limits imposed by these results.[21]

That the observed methyl tin products did not arise by a methyl migration to tin provoked by the actual hydride attack in the hydride generation analysis was established as follows. Yeast cultures were grown under standard experimental conditions in the absence of any tin compounds so that growth rate was at a maximum. The normal amount of the tin species was then added and a hydride generation analysis was carried out immediately. If the movement of a methyl group to tin was in any way produced by the actual analysis then methyl tin products would have been expected under these circumstances, as the fast growing yeast would have been at its richest in the methyl groups it was (presumably) capable of transfering to tin. No methyl tin products were observed under these conditions, we believe because the methylation is an ambient rate biologically induced process not a rapid hydride induced chemical transfer.

The importance of the work seems to us to be as follows: this is the first report on tin (II) methylation where full identification of methyl tin (IV) products has been made; it is the first report of a tin methylation by a pure strain of methylating species not obtained from an environmental source, and it is the first report where estimations of the yield and extent of a tin methylation are made. As a study made on a quantifiable and reproducible system it establishes tin methylation firmly as a biological process resulting from metabolic activity in a living organism. (Methylations from mixed systems are uncertain in that the proximate source of the transferred methyl group is not known; it may even be a chemical methylating species derived from local environmental pollution.) This work therefore establishes the reality of tin methylation under normal environmental conditions and its feasibility in the natural environment.

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REFERENCES

1. See for example Craig, P J, *In Organometallic Compounds in the Environment*, Craig, P J (ed) 1986, 65
2. Ridley, W P, Dizikes, L J and Wood, J M *Science*, 1977, 197: 329
3. Challenger, F. In: *Organometals and Organometalloids, Occurrence and Fate in the Environment*, Brinckman, F E and Bellama, J M (eds) ACS Symp. Ser. No. 82, Washington DC, 1978, 1
4. Reference 1, 139, 178, 212, 345, 1986
5. Braman, R S and Tompkins, M A *Anal. Chem.*, 1979, 51: 12
6. Hodge, V F, Seidel, S L and Goldberg, E D *Anal. Chem.*, 1979, 51: 1256
7. Jackson, J-A A, Blair, W R, Brinckman, F E and Iverson, W P *Environ. Sci. Technol.*, 1982, 16: 110
8. Andreae, M O and Byrd, J T *Science*, 1982, 218: 565.
9. Chau, Y-K, Wong, P T S and Bengert, G A *Anal. Chem.*, 1982, 54: 246
10. Blunden, S J and Chapman, A Reference 1, 1986, 117
11. Davies, A G and Smith, P J In: *Comprehensive Organometallic Chemistry*, Wilkinson, G, Stone, F G A and Abel, E W (eds) 1982, vol. 2, 584
12. Maguire, R J *Environ. Sci, Technol.*, 1984, 18: 291
13. Thayer, J S Inorg. Chem., 1979, 18: 1171
14. Hallas, L E, Means, J C and Cooney, J J *Science*, 1982, 215: 1505
15. Craig, P J Reference 1, 1986, 6
16. Craig, P J and Rapsomanikis, S *Inorg. Chim. Acta*, 1985, 197: 39
17. Craig, P J and Rapsomanikis, S *Environ. Sci. Technol.*, 1985, 19: 726
18. Thompson, J A J, Sheffer, M G, Pierce, R C, Chau, Y K, Cooney, J J, Cullen, W R and Maguire, R J *Organotin Compounds in the Aquatic Environment*, NRC Canada, 1985, 67
19. Cusack, P A, Smith, P J and Donaldson, J D *J. Chem. Soc. Dalton*, 1982, 439
20. Challenger, F In: Reference 3, pp. 2, 1978.
21. Ashby, J A and Craig, P J *Applied Organometallic Chemistry*, 1986, in press