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The methylation of inorganic tin by humic materials in an aquatic environment

Dai Shugui,* Huang Guolan and Cai Yong
Department of Environmental Science, Nankai University, Tianjin, People's Republic of China

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This paper presents a study of methylation of inorganic tin ($SnCl_4 \cdot 5H_2O$) by humic materials (humic and fulvic acids) isolated from the sediment of Tianjin Harbor, Tianjin, China, and the effects of pH, salinity, and the concentration of inorganic tin on the production of methyltin were investigated.

These humic materials could methylate inorganic tin, and the methyltin product was mainly monomethyltin. Low molecular weight compounds of the humus fraction (i.e. fulvic acid) were more active in the methylation, which could be facilitated by salinity and affected by pH.

Keywords: Humic material, methylation, methyltin, environmental sediment

INTRODUCTION

The bio- or nonbio-methylation of inorganic tin is an important pathway for the transportation and transformation of tin in the environment. Research in this field draws attention owing to the extensive application of organotin compounds, and a number of studies have been conducted. However, research in this area is just beginning in China.

It has been indicated by investigation that inorganic tin can be methylated in the aquatic environment if appropriate methyl donors exist. There are abundant humic materials in various waters and these substances can serve as methylating agents because they may contain methyl donors. Methylation of inorganic mercury by humic substances has been confirmed by the experiments of Nagase *et al.*[1] and Weber *et al.*,[2] but the role that humic substances play in methylation has not been reported yet.

This paper presents a study of methylation of inorganic tin ($SnCl_4 \cdot 5H_2O$) by humic substances (humic and fulvic acids) isolated from the sediment of Tianjin Harbor, Tianjin, China, and the effects of pH, salinity, and the concentration of inorganic tin on the methylation were investigated. It provides new evidence for a chemical methylation of inorganic tin in the environment.

EXPERIMENTAL

Isolation of humic acid (HA) and fulvic acid (FA)

The sediment used in this study was collected at Tianjin Harbor, and was dried at room temperature and then processed with a 60-mesh sieve. The portion of less than 60 mesh was used. The isolaton method can be seen in Ref. 3.

The extracting solution was a mixture of 0.2 mol dm^{-3} NaOH and 0.2 mol dm^{-3} $Na_4P_2O_7$. To 45 g sediment in a 500-cm^3 iodine flask, 150 cm^3 extracting solution was added. After shaking for 20 min, the flask lay still for four days. In the meantime, it was shaken 2–3 times a day, for 10 min each time. Then the mixture was put into two centrifuge tubes and centrifuged for 15 min. The upper solution was poured into a 250-cm^3 conical flask and the residue was discarded. The solution was acidified to about pH 3 with 1 mol dm^{-3} HCl, shaken for one more hour and centrifuged for 15 min. The upper solution was poured into a 250-cm^3 conical flask for use. The residue was mainly HA; this was dried at room temperature for further use. The FA solution was

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* Author to whom correspondence should be addressed.

adjusted to pH 7 with 1 mol dm^{-3} NaOH, and then dried using a water bath on a glass evaporating dish.

Reagents

These are noted below as follows:

Standard solution

Analytical grade $SnCl_4 \cdot 5H_2O$ (14.77 g) was dissolved in 100 cm^3 10% (v/v) HCl solution which contained 5% citric acid. The concentration of this standard solution was therefore 50 mg Sn cm^{-3}.

Grignard reagent

BuMgBr was synthesized in our laboratory with THF as solvent.

Extracting solution

This was 0.5% tropolone in benzene.

Reaction between humic materials and inorganic tin

The reactions were conducted in colorimetric tubes. The appropriate quantity of HA or FA and inorganic tin was added. The reaction conditions such as pH and salinity were adjusted and the reaction was carried out at room temperature in the dark for a given time. Samples were then taken for analysis (detailed procedures can be found in the following text).

Analysis of methyltin compounds

After butylation pretreatment, the samples were analyzed by a GC AA system. The instrumental conditions were as follows.

Gas chromatography

Glass column 3 mm × 2 m; 3% OV-1 on Chromosorb W AW-DMCS (80–100 mesh); high purity nitrogen as carrier gas with column pressure 3.0 kg cm^{-2}; (3.0×10^5 Pa); injection port temperature 170°C; column temperature program initially at 65°C, ascending to final 195°C at the rate of 40°C min^{-1}.

Atomic absorption spectrometry

Tin atomic lamp; wavelength 224.6 nm; lamp current 8 mA; transfer line temperature 170°C; quartz furnace temperature 850°C; hydrogen flow rate 118 cm^3 min^{-1}; air flow rate 56 cm^3 min^{-1}.

RESULTS AND DISCUSSION

Incubation time

Humic acid

Three 50-cm^3 colorimetric tubes were labeled A,B,C.

(a) Taking A as HA blank; 40 mg HA was added to the tube and this was diluted with 10% HCl to 25 cm^3 and then further diluted to 50 cm^3 with deionized water.

(b) Taking B as inorganic tin blank; 1 cm^3 standard solution was added to B, which was diluted with 10% HCl to 25 cm^3 and finally to 50 cm^3 with deionized water.

(c) Taking C as sample, 40 mg HA and 1 cm^3 standard solution were added to C, and the other procedure was the same as above.

The concentration of inorganic tin in B and C was 1 mg cm^{-3}. All experiments described above were duplicated.

After addition of the reagents, the solutions were placed in a high-pressure vessel at 1 atm (10^5 Pa) for sterilization and then incubated at room temperature in the dark. Samples (5 cm^3) of these solutions were tested periodically as follows. The sample was added to a 125-cm^3 separating funnel and 50 cm^3 deionized water, the pH value was adjusted to 5 with 1 mol dm^{-3} HCl and 1 mol dm^{-3} NaOH and then 19 g extra-pure NaCl and 5 cm^3 extracting solution were added. After shaking, the solution was allowed to stand for a certain time and the organic layer into which 1 cm^3 BuMgBr Grignard reagent was added was separated. After 15 min the excess Grignard reagent was destroyed with 1 mol dm^{-3} H_2SO_4. The organic layer was separated again and anhydrous sodium sulfate was added to absorb water. It was then injected into the GC AA instrument for analysis. The results are shown in Fig. 1 and Table 1.

Fulvic acid

The procedure of incubation and analysis was the same as that for HA; 45 cm^3 FA solution was added. Blanks were run at the same time and duplicated tests were performed for each experiment. The results are shown in Fig. 1 and Table 2; from them, it could be concluded that both HA and FA could react with Sn(IV) to produce CH_3Sn^{3+}, the amount of which increased with time, and it was relatively easier for FA to react with Sn(IV) than HA. It has been

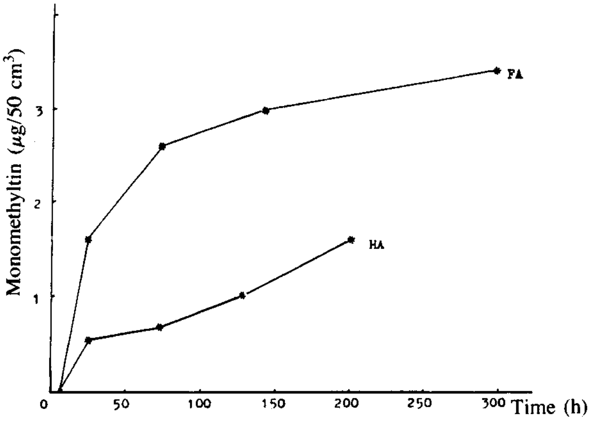

Figure 1 Monomethyltin production with time (HA and FA).

Table 1 Monomethyltin production with time (HA)

Time (h)	6	24	72	127	200
CH_3Sn^{3+} (μg/50 cm^3)	0	0.55	0.65	1.00	1.70

Table 2 Monomethyltin production with time (FA)

Time (h)	6	24	74	127	200
CH_3Sn^{3+} (μg/50 cm^3)	0	1.6	2.6	3.0	3.4

reported[4] that the structures of FA and HA are similar, and the differences between them are molecular weight, elemental composition, and the content of functional groups. The molecular weight of FA is relatively lower and a unit weight of FA may provide more methyl donors. In other words, FA is more active in methylation. Additionally, the incubation was conducted in an acidic environment and the solubility of HA was small. That may be one reason why CH_3Sn^{3+} produced was less for HA compared with FA.

Effect of pH

Artificial seawater was then selected as an incubation matrix to investigate the effect of pH on methylation. The artificial seawater was prepared according to Kesfer's formulation ‰ per mil (salinity $S = 35$‰); pH values were 2,4,6,8,10. Blank and duplicate tests were conducted under various conditions. HA (4 mg) or FA (2 mg) was added to the sample and other procedures were the same as above. The analytical result is shown in Fig. 2 and Table 3.

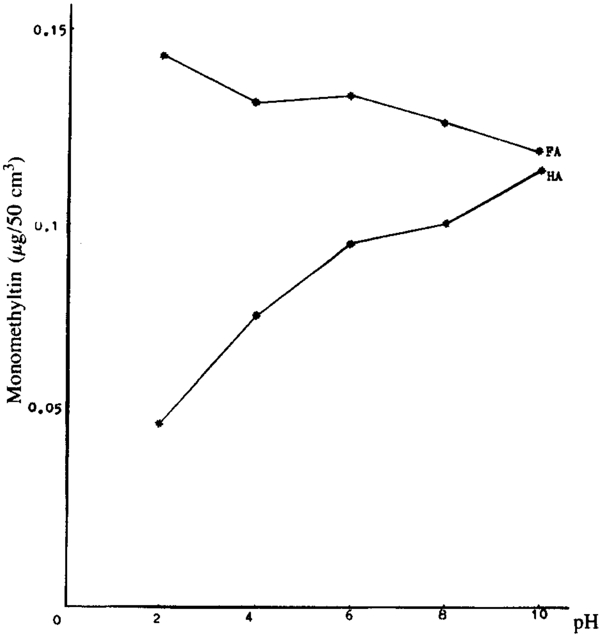

Figure 2 Monomethyltin production in HA and FA at various pH values.

Table 3 Monomethyltin production in HA and FA at various pH values

	CH_3Sn^{3+} (μg/50 cm^3)				
	pH 2	pH 4	pH 6	pH 8	pH 10
HA	0.048	0.076	0.095	0.100	0.114
FA	0.143	0.131	0.133	0.126	0.119

It can be seen from the data obtained that the amount of CH_3Sn^{3+} produced increased with pH in the HA incubation system, but the contrary result was found for FA solution. Under the experimental conditions, more homogeneous FA solution was formed but HA could not be dissolved completely and revealed a suspension. The solubility of HA increases with pH; this may be one cause for the larger amount of CH_3Sn^{3+} produced at higher pH in HA suspension. These results suggested that the reaction between inorganic tin and FA or HA could be affected by pH and that the effects were different on FA solution and HA suspension.

Salinity

Under simulated harbor conditions, experiments were conducted at pH 7.5, salinity 8,15,22,28,35‰ and

HA and FA blanks were also run at various salinities. The procedure of incubation and analysis was the same as above. Results are shown in Fig. 3 and Table 4.

The results showed that methylation was affected by the salinity of the matrix, and CH_3Sn^{3+} was produced more at higher salinity than at lower salinity. The conclusion was applicable to both FA and HA. It is known that humic substances can form complexes with metal ions, including inorganic tin. Under the experimental conditions of this study, inorganic anions, such as Cl^-, HCO_3^-, SO_4^{2-}, Br^-, etc. will compete with humic substances to form complexes. It is clear that competition is more severe at $S = 35$°/oo than at $S = 8$°/oo. Therefore, we can make the judgement that the inorganic tin anion complexes in seawater may more easily accept methyl groups supplied by humic substances and thus methylation is facilitated. This may be one reason why more CH_3Sn^{3+} is produced at higher salinity. In addition, CH_3Sn^{3+} production is apparently greater in FA solution than in HA suspension. This observation is compatible with the pH experiments, and incubation times, for which a partial reason has been discussed earlier.

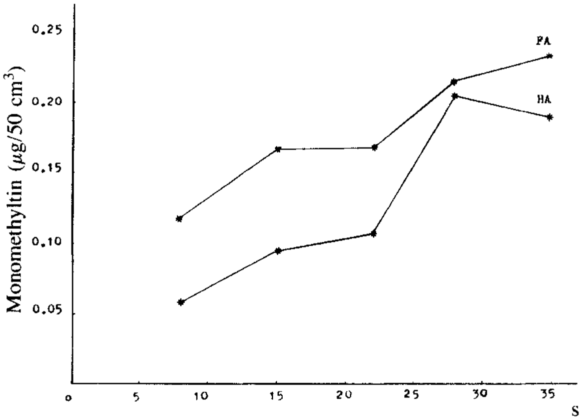

Figure 3 Monomethyltin production in HA and FA at various salinity values.

Table 4 Monomethyltin production in HA and FA at various salinity values

	CH_3Sn^{3+} (μg/50 cm³)				
S(°/oo)	S=8°/oo	S=15°/oo	S=22°/oo	S=28°/oo	S=35°/oo
HA	0.060	0.095	0.107	0.207	0.190
FA	0.119	0.167	0.167	0.216	0.233

Concentration of inorganic tin

Methyltin production at various inorganic tin concentrations was investigated separately at pH 7.5 with FA concentration 3 mg/50 cm³, and HA 15 mg/50 cm³. Other procedure was the same as above. The results are shown in Table 5 and Fig. 4. From Fig. 4, it can be found that initially, CH_3Sn^{3+} production increases with inorganic tin concentration, but tends to decrease when Sn(IV) concentrations go over a certain value. In the course of the experiment, a white precipitate was revealed gradually in the incubation tube with increasing inorganic tin concentration. This was due to hydrolysis of Sn(IV), and insoluble $Sn(OH)_4$ might have been formed, which would bar the methylation of inorganic tin. However, the large quantity of humic substances and inorganic complex ions in the matrix can form complexes with Sn(IV) within a range of inorganic tin concentrations, and thus the methylation was facilitated in this way.

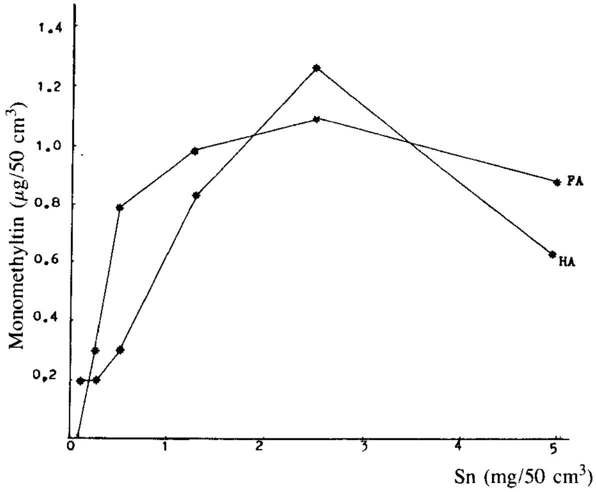

Figure 4 Monomethyltin production in HA and FA amended with inorganic tin of various concentrations.

Table 5 Monomethyltin production in HA and FA amended with inorganic tin of various concentrations

	CH_3Sn^{3+} (μg/50 cm³) Inorganic tin (mg/50 cm³)					
	0.10	0.25	0.50	1.25	2.50	5.00
HA	0.20	0.20	0.30	0.84	1.28	0.64
FA	0.00	0.30	0.79	0.99	1.09	0.89

From the results of the above experiments, it can be seen that humic substances isolated from harbor sediments really can methylate inorganic tin and that the product is mainly monomethyltin. Our previous investigations concerning organotin occurrence in Tianjin Harbor had shown that monomethyltin was the main methyltin species existing in that water body.

In general, there are a great number of organic compounds contained in the humic substance. It has been reported[1] that low-molecular-weight fatty acids, such as acetic acid, propionic acid, etc., could be methylating agents for mercury, but further investigation is needed to make sure whether these compounds can methylate inorganic tin or not. Nagase[1] has indicated that low-molecular-weight compounds had higher methylating activity for mercury. Our results suggested a similar observation for tin. Actually the methylation reaction taking place in this study was a *non*-biomethylation type. Numerous organic compounds were contained in the humic substances we used and a methylation reaction may exist as long as they can provide methyl groups. However, what compound brings about the methylation is still not known.

Weber *et al.*[2] have put forward three possible pathways for methylation on the basis of the methylation of mercury by soil fulvic acid (SFA). These pathways are: (1) nucleophilic attack by molecules which supply carbon anions (CH_3^-); (2) electrophilic attack by molecules which supply carbon cations (CH_3^+); and (3) mercury compound attack on methyl free radical donors. This prediction is helpful to further study on the methylation mechanism of inorganic tin by humic substances.

CONCLUSION

Firstly, humic substances (FA and HA) isolated from Tianjin Harbor, Tianjin, China, can methylate inorganic tin ($SnCl_4 \cdot 5H_2O$), and the methylated product is mainly monomethyltin (CH_3Sn^{3+}); secondly, FA is more active than HA in the methylation of tin; and thirdly, high salinity facilitates the methylation which can also be affected by pH.

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