Bull. Environm. Contam. Toxicol. 23,848-854 (1979)

Non-Enzymatic Reduction of Organomercurial Salts in Biological Systems

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Mercury redox transformation reactions in nature may be biological (enzymic) or non-biological (non-enzymic). Biological mercury transformation has usually been explained in terms of the action of a number of specialized enzymes (DE FILIPPIS 1978, IZAKI et al. 1974, SCHOTTEL et al. 1974). While non-biological transformation has been explained in terms of the action of certain biological products such as methylcobalamin (methyl vitamin B_{12}), methionine, ascorbate, humic acid and some proteins (ALBERTS et al. 1974, BERTILSSON & NEUJAHR 1971, IMURA et al. 1971, LANDLER 1971). DE FILIPPIS & PALLAGHY (1975) have already shown that mercuric salts (Hg^{++}) can be reduced and alkylated by the action of ethylene and acetylene (two known reducing agents in air), and STRINI & METZGER (1966) have also established that mercuric salts are reduced by propene and other higher molecular weight unsaturated hydrocarbons (olefins).

Although non-biological transformation reactions involving mercuric salts have been studied, similar types of reactions involving organomercurial compounds have not. The biological products ethylene (C_2H_4) and acetylene (C_2H_2) are known reducing agents and should also readily reduce, for example, phenylmercuric salts from phenylmercuric acetate (PMA) solutions. The possible involvement of ethylene and acetylene in a mechanism related to mercury transformation, and possibly tolerance in living organisms may have been ignored because of the low concentrations of these gases in the environment (0.05 to 1.0 µL/L of air). However the data given below show that metallic mercury can be produced and vaporized from 0.5 µM solutions of PMA either by the action of air, or by a purified airstream containing 1.0 µL/L (1.0 ppm) of ethylene and acetylene. The reaction is light mediated, pH dependent, inhibited by 20 µM $AgNO_3$ and 3 M Na_2S, but is largely independent of oxygen concentration. Therefore unsaturated hydrocarbons play an important role certainly in the reduction, and possibly also the alkylation of

0007-4861/79/0023-0848 $01.40

mercury in biological systems, and the environment in general.

MATERIALS AND METHODS

Mercury (^{203}Hg) volatilization was tested by adding [^{203}Hg] PMA to either distilled water or an inorganic culture medium (ICM) which was normally used for growing the alga Chlorella vulgaris (DE FILIPPIS & PALLAGHY 1976). Solutions at pH 5.0 and 8.0 were prepared by the addition of a phosphate buffer.

Solutions were autoclaved and, in selected experiments were demonstrated to be free of microorganisms by plating samples before, and after each run on bacterio-agar. [^{203}Hg] PMA was added to 50 mL of solution, which had been previously purged with the appropriate gas stream. Except for a 4 cm long Tygon connection, an all glass system was used and a flow rate of 100 mL of gas/min was maintained. We used glass enclosed magnetic stirrers and found that less than 1% of the PMA was absorbed on to the surface of the vessels (DE FILIPPIS & PALLAGHY 1975). Aqua regia traps were used for the trapping and detection of mercury volatile products (KOMURA & IZAKI 1971).

In the absence of a suitable gas chromatograph, we attempted to identify the species of mercury evolved by using three traps placed in series in the following order: carbonate-phosphate absorption solution for methyl and ethyl mercury; acid permanganate for collection of elemental mercury; and gold foil for other organomercurials (HENRIQUES et al. 1973). ^{203}Hg was measured using liquid scintillation.

RESULTS AND DISCUSSION

When the incoming air was scrubbed with alkaline permanganate ($KMnO_4$), which removes traces of reducing agents and unsaturated organic compounds, the percentage of ^{203}Hg lost from the ICM or water was greatly reduced. This loss of mercury was also dependent on whether illumination was provided or not (Table 1).

Further results, summarized in Table 2 (and also Figure 1) indicate that 1.0 µL/L of ethylene or acetylene volatize mercury from solutions; which could be quantitavely recovered in the traps (Table 3). Additional experiments showed that 20 µM $AgNO_3$ and 3 M Na_2S were able to greatly reduce the ethylene and acetylene mediated evolution of mercury (Table 2). Raising the pH markedly enhanced the induced mercury evolution (Table 1), suggesting that the known

TABLE 1

^{203}Hg lost over the first 12 h from 0.5 μM [^{203}Hg]-PMA in either distilled water or inorganic culture medium (ICM) at pH 5.0 or 8.0 and 25°C. Quantum flux from an incandescent light source was 16 μE $m^{-2}s^{-1}$ (680 lux). Average of four determinations.

	Medium	pH	% ^{203}Hg volatilized	
			Laboratory air	$KMnO_4$-scrubbed air
Dark	ICM	5.0	9.4	3.5
		8.0	9.8	4.3
	H_2O	5.0	8.9	4.2
		8.0	10.2	4.6
Light	ICM	5.0	28.5	8.0
		8.0	39.4	8.6
	H_2O	5.0	29.2	7.3
		8.0	38.9	8.5

TABLE 2

Effect of ethylene, acetylene, $AgNO_3$ and Na_2S on ^{203}Hg lost over 12 h into various air streams from 0.5 μM [^{203}Hg] PMA in the ICM (pH 5.0) at 25°C. Quantum flux was 16 μE $m^{-2}s^{-1}$. Average of four determinations.

Hydrocarbon in airstream (μL/L)	Compounds added	% ^{203}Hg volatilized	
		N_2	21%O_2/79%N_2
None	—	4.1	4.5
	20 μM $AgNO_3$	3.8	4.2
	3 M Na_2S	3.9	4.4
C_2H_4 (1.0)	—	28.2	28.5
	20 μM $AgNO_3$	8.5	7.5
	3 M Na_2S	10.5	11.2
C_2H_2 (1.0)	—	29.8	28.6
	20 μM $AgNO_3$	7.6	8.0
	3 M Na_2S	11.0	10.1

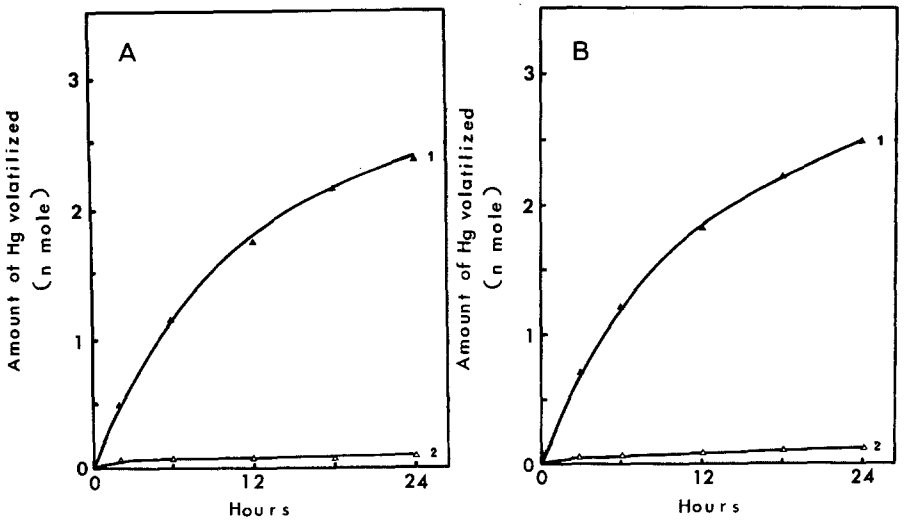

FIGURE 1: Time course for the evolution of mercury from PMA solutions at pH 5.0 and at 25°C. The total amount of PMA available for reduction was 7.5 nmol. Quantum flux was 16 μE $m^{-2}s^{-1}$. Average of three determinations.

A: (1) N_2+1.0 μL/L C_2H_4; (2) N_2 only.
B: (1) N_2+1.0 μL/L C_2H_2; (2) N_2 only.

TABLE 3

Mercury volatilized (± standard deviation) over the first 12 h which was recovered in each of three traps. ^{203}Hg was lost from 0.5 μM [^{203}Hg] PMA in the ICM at 25°C and under ordinary laboratory air. Quantum flux was 16 μE $m^{-2}s^{-1}$.

Vapour trap	% recovery of the ^{203}Hg volatilized (±S.D.)
Carbonate phosphate	7.8 ± 1.3
Acid permanganate	88.7 ± 3.0
Gold foil	2.6 ± 2.5

instability of weakly acid or alkaline mercury solutions may be due to this effect.

The reason why lowering the pH can prevent some mercury from volatilizing is that under high oxidizing conditions (i.e. low pH), the equilibrium between ionic and metallic mercury is shifted towards the ionic mercury state, and therefore more of the mercury is retained in solution. Silver ions (Ag^{+}) are strong oxidizing agents and can also compete with the phenylmercuric ion for reduction, this could explain why Ag^{+} almost completely inhibits mercury loss. LO & WAI (1975) have shown that auric (Au^{+++}) ions also prevent mercury loss from $Hg(NO_3)_2$ solutions; Au^{+++} are also powerful oxidizing agents. Excess sulphide ions (S^{--}) apparently bind the phenylmercuric ions sufficiently to prevent them from being reduced. The reaction between ethylene or acetylene and PMA solutions are complex and do not follow simple first or second order kinetics.

The loss of mercury from PMA solutions would be in agreement with the fact that organomercurials have a greater volatility than mercuric ions (KOTHNY (1972). BURROWS & KRENKEL (1974) reported that this is the mechanism by which mercury is lost from methylmercuric chloride solutions. But in our case we observed that over 85% of the volatilized mercury was in the acid-permanganate trap (Table 3), suggesting that most of the volatile mercury was in the metal form (Hg^{o}). In aqueous solutions the phenylmercuric ions are very unstable and the phenyl group readily splits off to form benzene and mercuric ions (GAVIS & FERGUSON 1972); Hg^{++} can then be reduced to the metallic form by the action of ethylene and acetylene (DE FILIPPIS & PALLAGHY 1975).

The mechanism of mercury volatilization has been a subject of much controversy, especially since some workers (ALBERTS et al. 1974, IZAKI et al. 1974, KOMURA & IZAKI 1971, SCHOTTEL et al. 1974, TONOMURA et al. 1968, TORIBARA et al. 1970) have invoked bacterial (as contamination) action as a means of converting inorganic mercuric ions to the more volatile metallic or organic forms of mercury. However, wherever contamination by microorganisms is absent, as was the case in these experiments, an alternate mechanism must be present. Some authors (TORIBARA et al. 1970) have suggested that mercury solutions commonly found in the laboratory are usually contaminated with sufficient reductants to carry out such redox reactions. These reductants we suggest might be ethylene, acetylene or any other unsaturated hydrocarbon.

There are a number of striking similarities

between ethylene and acetylene mediated mercury volatilization and some features of mercury transformation reactions in biological systems. Firstly, TONOMURA et al. (1968) suggested as a possibility that the vaporization of PMA by mercury resistant bacteria may involve a gaseous substance evolved from the bacterial surface. Secondly, DE FILIPPIS & PALLAGHY (1976) have already shown that ethylene evolved from the green alga Chlorella was responsible for some of the reduction and volatilization of mercury from the culture medium, but an enzyme system was also involved. Thirdly, mercury volatilization by mercury resistant organisms, from which a mercury reducing enzyme has been isolated is also markedly inhibited by Ag^{+} (DE FILIPPIS 1978, IZAKI et al. 1974, SCHOTTEL et al. 1974). Fourthly, plants, bacteria and fungi may produce considerable amounts of ethylene (LYNCH 1975, SEQUEIRA 1973). Fifthly, methylation of mercury by Neurospora involves methionine biosynthesis (Landler 1971), which is the probable pathway for ethylene biosynthesis in plants (YANG 1968).

It seems likely, therefore, that any one of a number of unsaturated hydrocarbons, ethylene in particular, may be involved in the non-biological reduction or conversion of mercury compounds. It has already been noted by SUMMERBELL et al. (1962), amongst others, that whether one obtains the alkylated or reduced metallic form of mercury in reactions of mercuric ions with an olefin depends simply on the mole ratio of reagents. In view of this hypothesis, it would seem important for investigators to test whether mercury resistant strains of organisms also had a greater capacity to produce unsaturated hydrocarbons.

The likely reduction of mercury by low concentrations of ethylene and acetylene regardless of whether they originate from biological sources or atmospheric pollution, suggests the alternate mechanism (ALBERTS et al. 1974, SCHOTTEL et al, 1974) by which elemental mercury might be released into the atmosphere.

ACKNOWLEDGEMENTS

I thank G. Holden and M. Shaw for providing a motor driven gas syringe, C.K. Pallaghy for his interest and encouragement, and the receipt of a Commonwealth Scholarship.

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