A Simple Model for the Non-Enzymatic Reduction and Alkylation of Mercuric Salts in Biological Systems

by L. F. De Filippis and C. K. Pallaghy
Department of Botany, La Trobe University
Bundoora 3083, Victoria, Australia

Redox transformations of mercurial compounds in nature have been explained in terms of actions of either specialized enzymes or certain biological products such as methyl cobalamin and humic acid (ALBERTS et al. 1974, KRENKEL 1974, VALLEE and ULMER 1972). However, even though the formation of methyl mercury as a bi-product in the industrial preparation of acetaldehyde by the hydration of acetylene is well known (KRENKEL 1974), no attention has yet been given to the possibility that the biological products, ethylene and acetylene (SEQUEIRA 1973, YANG 1968), may be involved in a simple non-enzymatic reaction protecting organisms from exposure to mercuric ions.

Ethylene and acetylene are known reducing agents and should readily reduce mercuric solutions because of the extremely high potentials of the Hg^{2+}/Hg_2^{2+} and Hg^{2+}/Hg^{0} couples. It has already been established that mercuric salts in reactions with high concentrations of propene and other higher molecular weight olefins may yield metallic mercury (STRINI and METZGER 1966). The possible involvement of ethylene or acetylene in a mechanism related to mercury tolerance in living organisms may have been ignored because of the low concentrations of these gases in the environment (0.005 to 0.05 µl per liter of air). The data given below confirm that mercury may be vaporized from 1.5 µM solutions of $HgCl_2$ either by air, or by a purified airstream containing 0.1 µl per liter (0.1 ppm) of ethylene or acetylene. The reaction is light sensitive, pH dependent, inhibited by 20 µM $AgNO_3$, but is largely independent of oxygen concentration. It is hypothesised that unsaturated hydrocarbons may play an important role in the reduction or alkylation of mercury compounds in biological systems and in the environment in general.

METHODS AND MATERIALS

Mercury volatilization was tested by adding $[^{203}Hg]HgCl_2$

to either distilled water or an inorganic medium which we would
normally use for culturing the alga Chlorella vulgaris. The
inorganic medium had the following composition:

KNO_3 = 25 mM; $MgSO_4$ = 20 mM; KH_2PO_4 = 11 mM;

$FeCl_3$ = 10 µM; $ZnSO_4$ = 7.3 µM; H_3BO_3 = 16.2 µM;

$MnSO_4$ = 8.9 µM; $CuSO_4$ = 0.12 µM; $(NH_4)_6Mo_7O_{24}$ = 4 nM;

pH = 5.3

Solutions at pH 5 and 8 were prepared by addition of 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.
$[^{203}Hg]Hg\,Cl_2$ 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 per minute was
maintained (PALLAGHY and RASCHKE 1972). We used glass
enclosed magnetic stirrers and found that less than 1% of the
$HgCl_2$ was adsorbed on to the vessels (GREENWOOD and
CLARKSON 1973). An aqua regia trap was used for the
detection of mercury volatiles (KOMURA and 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
adsorption solution for collection of 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 $KMnO_4$,
which removes traces of reducing agents and unsaturated
organic compounds, the percentage of $^{203}Hg^{2+}$ lost from the
inorganic medium was reduced from 27.6% in air to 7.9% in
purified air over a twenty-four hour period. In distilled water,
the corresponding amounts were 27.4% and 4.7% respectively.
Subsequent studies showed that 10 µl per liter of ethylene or
acetylene added to $KMnO_4$-scrubbed air caused $^{203}Hg^{2+}$ losses
of 33.5% and 39% respectively over a similar period.

Further results, summarized in Table I as the arithmetic
means of several series of experiments, indicate that 0.1 µl

per liter of ethylene or acetylene volatilizes mercury from solution, which could be quantitatively recovered in the traps.

TABLE I

Percentage of ^{203}Hg lost over the first twelve hours from 50 ml of 1.5 µM$[^{203}$Hg$]$HgCl$_2$ in inorganic medium at 25°C. Quantum flux from an incandescent light source was approximately 16 µE m^{-2} s^{-1} (680 lux).

Hydrocarbon added (µl per liter)	pH of I. C. M. solution	Pure oxygen and nitrogen mixture (21% O$_2$/79% N$_2$)	High purity nitrogen
0	5.0	-	12.6%
0	5.3	15.7%	14.8%
0	8.0	-	16.8%
C$_2$H$_2$(0.1)	5.3	-	53.7%
C$_2$H$_4$(0.1)	5.3	44.7%	50.2%
C$_2$H$_4$(1.0)	5.0	-	38.6%
C$_2$H$_4$(1.0)	8.0	-	52.2%

Of the mercury evolved, 85% was in the KMnO$_4$ trap and the remainder in the gold foil, suggesting that most of the mercury evolved may have been in the elemental form (Hg^0). These percentages were identical whether air or ethylene added to KMnO$_4$-scrubbed air was used. However, these traps are not totally reliable indicators (HENRIQUES et al. 1973). The mercury evolution observed under ethylene- or acetylene-free conditions (Fig. 1, Table I) indicates trace amounts of reducing agents in the solution or that some mercury in the vials of $[^{203}$Hg$]$HgCl$_2$ supplied was already in the reduced form. The latter was more likely, since the amounts evolved were reproducible for samples taken from the one vial, but varied between vials.
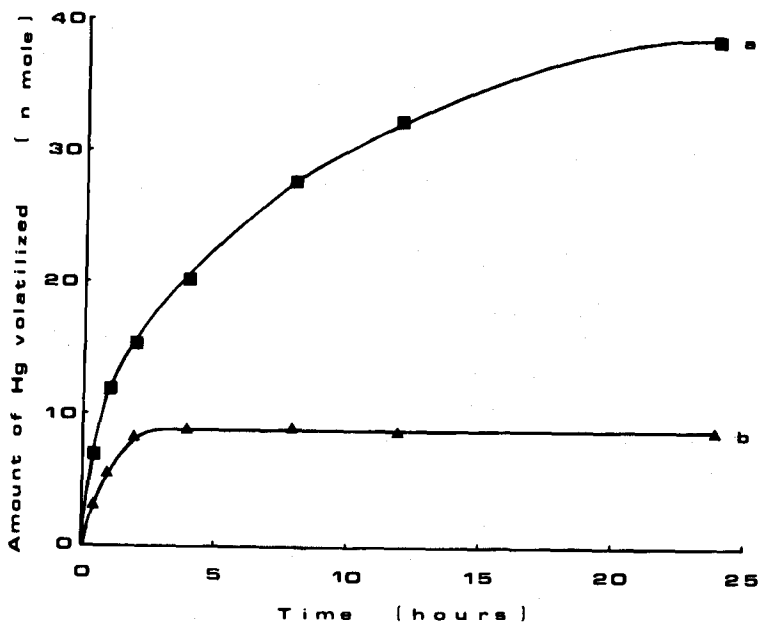
Figure 1. Evolution of mercury from inorganic medium plotted against time at pH 5.3. (a) 0.1 μl of ethylene per litre of high purity nitrogen (b) high purity nitrogen. The reactions do not follow first order kinetics. In each case, the total amount of $HgCl_2$ available for reduction was 75 n moles. Quantum flux was approximately 16 $\mu E\ m^{-2}\ s^{-1}$.

Additional experiments showed that darkness reduced the ethylene, acetylene, or air mediated evolution of mercury by about 30%, while 20 μM $AgNO_3$ inhibited it completely. $AgNO_3$ did not affect the residual component of mercury evolution observed in high purity nitrogen or $KMnO_4$-scrubbed air. Raising the pH markedly enhanced the ethylene induced mercury evolution (Table I), suggesting that the known instability of weakly acid or alkaline $HgCl_2$ solutions may be due to this effect.

There are a number of striking similarities between the data presented above and some of the features of mercury conversions in biological systems. First, TONOMURA et al. (1968) suggested as a possibility that the vaporization of phenylmercuric acetate by mercury resistant bacteria may

involve a gaseous substance evolved from the bacterial surface. Second, plants and a large number of bacteria and fungi may produce considerable amounts of ethylene (SEQUEIRA 1973). Third, mercury vaporization by an $HgCl_2$-resistant strain of E. coli, from which an Hg^{2+} reducing enzyme has been isolated (IZAKI et al. 1974), is also markedly inhibited by Ag^+ (SUMMERS and SILVER 1972). Fourth, methylation of Hg^{2+} by Neurospora involves methione biosynthesis (LANDLER 1971), which is the probable pathway for ethylene biosynthesis in plants (YANG 1968). Fifth, the methylation and reduction of mercury compounds by methanogenic bacteria is well known (WOOD et al. 1968). It seems likely, therefore, that any one of a number of unsaturated hydrocarbons, ethylene and acetylene in particular, may be involved in the 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 form of mercury in reactions of Hg^{2+} with an olefin depends simply on the mole ratio of the reagents. In view of this hypothesis, it would seem important for investigators to test whether Hg^{2+}-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 an alternate mechanism (ALBERTS et al. 1974) by which elemental mercury might be released into the atmosphere.

ACKNOWLEDGEMENTS

REFERENCES

ALBERTS, J. J., J. E. SCHINDLER and R. W. MILLER: Science 184, 895 (1974)

GREENWOOD, M. R. and T. W. CLARKSON: Amer. Ind. Hygiene Ass. 31, 250 (1973)

HENRIQUES, Å., J. ISBERG and D. J. KJELLGREN: Chimica Scripta 4, 139 (1973)

IZAKI, K., Y. TASHIRO and F. FUNABA: J. Biochem. 75, 591 (1974)

KOMURA, I. and K. IZAKI: J. Biochem. 70, 885 (1971)

KRENKEL, P. A.: Crit. Rev. Environ. Control 4, 251 (1974)

LANDLER, L.: Nature 230, 452 (1971)

PALLAGHY, C. K. and K. RASCHKE: Plant Physiol. 49, 275 (1972)

SEQUEIRA, L.: Ann. Rev. Plant Physiol. 24, 353 (1973)

SUMMERBELL, R. K., G. H. KALB, E. S. GRAHAM and A. L. ALLRED: J. Org. Chem. 27, 4461 (1962)

SUMMERS, A. O. and S. SILVER: J. Bacteriol. 112, 1228 (1972)

STRINI, J. -C. and J. METZGER: Bull. Chem. Soc. France 10, 3150 (1966)

TONOMURA, K., K. MAEDA, F. FUTAI, T. NAKAGAMI and M. YAMADA: Nature 217, 644 (1968)

VALLEE, B. L. and D. D. ULMER: Ann. Rev. Biochem. 41, 91 (1972)

WOOD, J. M., F. S. KENNEDY and C. J. ROSEN: Nature 220, 173 (1968)

YANG, S. F.: in Biochemistry and Physiology of Plant Growth Substances.
Editors WIGHTMAN, F. and G. SETTERFIELD, Ottawa, Runge Press, 1968, p. 1217