22 July 1977, Volume 197, Number 4301 SCIENCE

Biomethylation of Toxic Elements in the Environment

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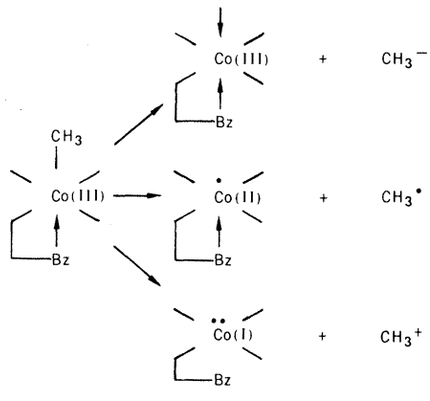
Biomethylation of toxic elements can be shown to occur by three alternative mechanisms. Until recently it was not understood how these mechanisms could be used to predict the environmental requirements for methyl transfer to toxic elements. On the basis of oxidation-reduction chemistry, we can now formulate the conditions for biomethylation and understand the important role played by oxygen and transition metal ions in the biomethylation process. In this article we examine mechanisms for methyl transfer to mercury, palladium, thallium, lead, platinum, gold, tin, chromium, selenium, arsenic, and sulfur. We also show how the oxidation-reduction chemistry of these elements provides us with information about the environmental conditions required for the biomethylation of each element.

Vitamin B_{12}–Dependent Reactions

The biotransformation of toxic substances in the environment is of vital importance from the standpoint of public health since it is the molecular form of these species which controls their persistence, bioaccumulation, and toxicity. The conversion of inorganic mercury in the form of Hg(II) to the potent neurotoxin methylmercury by bacteria and molds is an example of one such transformation whose consequences are extremely dangerous (*1–3*). A survey of the methylating agents which are available for methyl transfer in biological systems reveals that three major coenzymes are known to be involved in this reaction: (i) *S*-adenosylmethionine, (ii) N^5-methyltetrahydrofolate derivatives, and (iii) vitamin B_{12} (methylcorrinoid) derivatives.

The methylcorrinoid derivatives are believed to be the methylating agents for inorganic mercury salts, since they are the only agents known to be capable of transferring carbanion methyl groups (CH_3^-). Both *S*-adenosylmethionine and N^5-methyltetrahydrofolate donate carbonium methyl groups (CH_3^+) (*4*). Methylcobalamin has been implicated in the methylation of a number of metals including Pb, Sn, Pd, Pt, Au, and Tl as well as the metalloids As, Se, Te, and S (*3–7*). Theoretically, there are three mechanisms which may be involved in the methylation of these metals and metalloids, since methylcorrinoids are capable of transferring methyl groups as radicals ($CH^{\bullet}$) or carbonium ions as well as carbanions, as shown in scheme I.



Bz = 5,6-dimethylbenzimidazole

We have shown that carbanion and radical transfer reactions are the predominant mechanisms for B_{12}-dependent methylation of metals and metalloids (*8–10*). However, it is necessary to have a general theory for predicting which mechanism operates for a particular element if we are to predict environmental conditions for biomethylation.

The oxidation state of an element has been shown to be important in the methylation of metals or metalloids by methylcobalamin. For example, mercuric acetate reacts very rapidly with methylcobalamin to produce methylmercury, but if the reaction is run in the presence of ascorbate, which reduces Hg(II) to Hg_2(II), there is no observed reaction (*11*).

To date, we have found two general mechanisms for the methylation of a metal or metalloid by methylcobalamin: (type 1) reactions in which the metal or metalloid acts as an electrophile and (type 2) reactions in which the metal or metalloid acts to abstract a methyl radical. Reactions of type 1 involve heterolytic cleavage of the Co–C bond of methylcobalamin with the transfer of a carbanion methyl group to the more oxidized state of the element. Type 2 reactions involve homolytic cleavage of the Co–C bond with methyl radical transfer to the reduced member of a redox couple.

The reaction between mercuric acetate and methylcobalamin is an example of type 1 (Fig. 1). A carbanion methyl group is transferred to mercury to give CH_3Hg^+, and a water molecule coordinates in the fifth ligand site of the cobalamin to give aquocobalamin [Co(III)] as a final product. Because Hg(II) is a good electrophile, it also coordinates with the nitrogen of the 5,6-dimethylbenzimidazole moiety of methylcobalamin to give a mixture of "base-on" and "base-off" species. The base-on species has been shown to react with Hg(II) at least 1000 times faster than the base-off species (*11*). This difference in reaction rate is presumably due to the difference in electron density at the Co–C bond; the base-on species has a greater electron density because of the coordination of the benzimidazole nitrogen in the sixth ligand site. Other metals which are known to

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react with methylcobalamin in a type 1 reaction are Pd(II) (*12*) and Tl(III) (*13*).

Type 2 reactions involve reductive homolytic cleavage of the Co–C bond of methylcobalamin with the transfer of $CH_3\cdot$ and the production of Co(II) cobalamin. The attacking metal or metalloid species couples with $CH_3\cdot$, resulting in a one-electron oxidation of the attacking element. An example of a type 2 reaction is the methylation of thiols by methylcobalamin. In the case of ethanethiol sulfonic acid, or mercaptoethanol, the reaction has been shown to involve thiol-free radicals (*14*). This methylation requires the presence of oxygen for the generation of the radical species and is characterized by a lag period during which a steady state concentration of attacking radicals is generated. A mechanism for this reaction is presented in Fig. 2. The methylation of Cr(II) by methylcobalamin appears to be of the type 2 class, but in this case no lag period is observed since the attacking species is Cr(II) (*15*). Cobalamin [Co(II)] and $CH_3Cr(H_2O)_5^{2+}$ are products of this reaction, but the later is rapidly cleaved under acid conditions to give methane and Cr(III).

The reduction potentials for the elements known to be methylated by methylcobalamin are shown in Table 1. It is apparent that elements which react by electrophilic heterolytic cleavage (type 1 mechanism) have reduction potentials greater than +0.8 volt. It is also evident that the more oxidized member of the redox couple—for example, Tl(III), Pd(II), or Hg(II)—is the species which is methylated by methylcobalamin. At the other end of the redox potential scale, the reducing end, are elements which are known to react by reductive homolytic cleavage (type 2 mechanism). For these elements the member of the redox couple in the low oxidation state—for example, the thiol or Cr(II)—is the species which reacts with methylcobalamin. The reduction potential of a couple is an indication of the relative thermodynamic tendency of the species involved to accept or donate electrons. Therefore, one might expect elements with a high reduction potential to be good oxidizing agents and to react by an electrophilic mechanism. Elements with a low reduction potential are, on a relative scale, better reducing agents, and it would seem logical that they would tend to react by a reductive mechanism.

Table 1. Relationship between standard reduction potential (E^0) (*28*) and the mechanism of methylation for selected elements.

Redox couple	E^0 (volts)	Mechanism of methylation
Pb(IV)/Pb(II)	+1.46	Type 1 (*9, 18*)
Tl(III)/Tl(I)	+1.26	Type 1 (*13*)
Se(VI)/Se(IV) acid	+1.15	
Pd(II)/Pd(0)	+0.987	Type 1 (*12*)
Hg(II)/Hg(0)	+0.854	Type 1 (*11*)
Au(III)/Au(I)	+0.805	Redox switch (*13*)
Fe(III)/Fe(II)	+0.771	
Pt(IV)/Pt(II)	+0.760	Redox switch (*13*)
As(V)/As(III) acid	+0.559	
Sn(IV)/Sn(II)	+0.154	Type 2 (*10*)
Se(VI)/Se(IV) base	+0.05	
Cys–S–S Cys/2Cys–SH	−0.22	Type 2 (*14*)
Cr(III)/Cr(II)	−0.41	Type 2 (*15*)
As(V)/As(III) base	−0.67	

When attempting to divide elements into those methylated by a type 1 and those methylated by a type 2 mechanism, the question of where to put the dividing line arises. In other words, is there a reduction potential above which a type 1 mechanism and below which a type 2 mechanism predominates? Examination of Table 1 indicates that there is a group of elements with reduction potentials between +0.559 and +0.805 volt which do not fit into our general classification. These potentials cluster around the value observed for oxygen

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 + 0.682 \text{ volt}$$

Agnes *et al.* (*13*) showed that for reactions with gold and platinum both oxidation states, Au(III)/Au(I) and Pt(IV)/Pt(II), are necessary for methylation to occur. These authors proposed a mechanism which accounts for the observed first-order dependence on both oxidation states and called it a redox switch.

Very few data have appeared in the literature regarding the reaction of methylcobalamin with two environmentally significant metals, tin and lead. These metals are members of group IVA of the periodic table. We have studied the methylation of tin in an attempt to determine whether this element reacts in a type 1, type 2, or redox switch mechanism. Our intention was to then examine lead and compare the results for these two members of group IVA. This would address the question of whether members of the same group in the periodic table react with methylcobalamin in the same manner.

The reduction potential of the Sn(IV)/Sn(II) couple, as shown in Table 1, is +0.154 volt. This is well below the reduction potential for O_2/O_2^{2-}, and there-

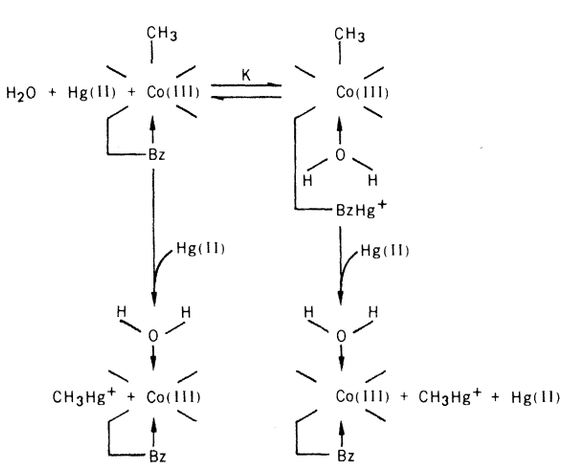
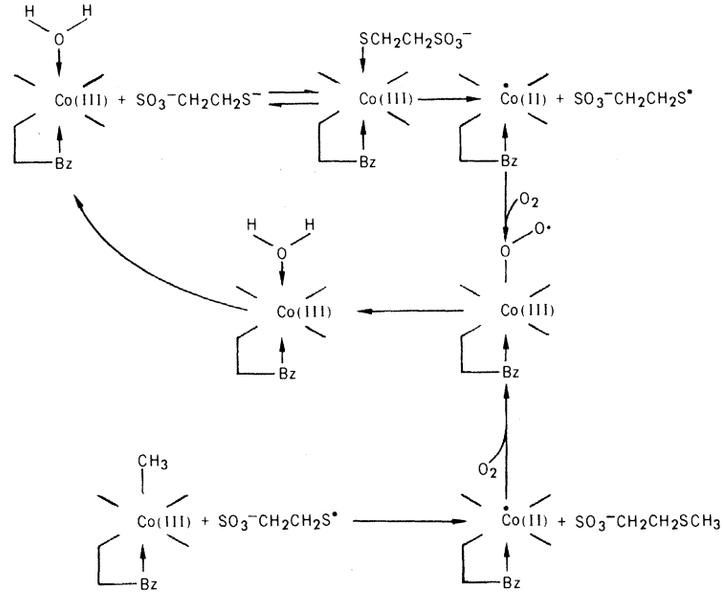


Fig. 1 (left). Mechanism for vitamin B_{12}–dependent methyl transfer to mercuric ion (type 1). Fig. 2 (right). Mechanism for vitamin B_{12}–dependent methyl transfer to thiols (type 2).



fore one would predict that tin should react by a type 2 mechanism. We showed recently that Sn(IV) does not react with methylcobalamin, but Sn(II) reacts if a single-electron oxidant such as Fe(III) or Co(III) is present (*10*). A detailed kinetic study of this reaction shows that biomethylation of tin does involve a type 2 mechanism (*10*) (Fig. 3).

The initial step in the reaction, when Fe(III) is used as the oxidizing agent, involves a one-electron oxidation of Sn(II) to Sn(III)·, a stannyl radical (Fig. 3). Under reaction conditions such that there is a high chloride ion concentration, a principal tin species in solution is $SnCl_3^-$, which, when oxidized, would give $SnCl_3$·, a species for which there is a chemical precedent (*16*). The stannyl radical, which is present in low concentration, then reacts in the rate-limiting step with methylcobalamin to give homolytic cleavage of the Co–C bond, affording the observed kinetics and producing CH_3SnCl_3 and reduced cobalamin [Co(II)]. Finally, in this system, the Co(II) is very rapidly oxidized to give the observed aquocobalamin [Co(III)] product. These observations provide a possible mechanism for the microbial methylation of tin, which has been found to occur in a *Pseudomonas* species isolated from Chesapeake Bay (*17*).

Examination of Table 1 indicates that the lead redox couple, Pb(IV)/Pb(II), has a much higher reduction potential than tin. The reduction potential theory would predict that because of this high potential, +1.46 volts, Pb(IV) should react electrophilically. Recent work by Taylor and Hanna (*18*) has shown that prolonged incubation of methylcobalamin with fine suspensions of Pb(IV) oxides results in partial demethylation of the corrinoid. Tracer studies with [^{14}C]methyl-labeled methylcobalamin indicated that demethylation with Pb(IV) was accompanied by a proportional volatilization of the label. These results are in agreement with the known lability of monoalkyl lead derivatives. Taylor and Hanna (*18*) observed no demethylation in the presence of a variety of Pb(II) salts, which agrees with our previous observations (*3*). We have also observed that dialkyl Pb(IV) salts result in a slow but perceivable demethylation of methylcobalamin (*9*). Together, all of these results suggest that lead reacts according to an electrophilic heterolytic (type 1) mechanism and that it is the higher oxidation state of the element which is important. So far we have been unable to isolate or synthesize a monoalkyl lead complex which is stable in water, although three laboratories have reported

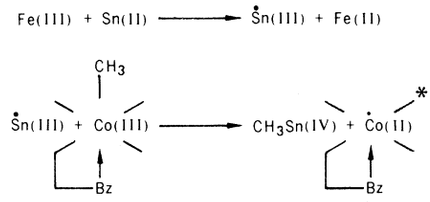


Fig. 3. Mechanism for vitamin B_{12}–dependent methyl transfer to tin (type 2). (*) Cobalt(II) is readily oxidized by O_2 to Co(III), as shown in Fig. 2. Cobalt(III) will substitute for Fe(III) in the oxidation of Sn(II) to Sn(III).

that inorganic lead salts can be methylated to tetramethyl lead by mixed microbial communities (*19–21*).

Two other considerations should be mentioned regarding the application of the reduction potential theory. An examination of Table 1 shows that for certain elements the reduction potential is a strong function of *p*H. Under acidic conditions the Se(VI)/Se(IV) redox couple has a potential, +1.15 volts, which would classify it for a type 1 mechanism. However, the potential is dramatically lowered under basic conditions to +0.05 volt. Under basic conditions, the theory would predict that Se(IV) should react by a type 2 mechanism.

A second consideration is the effect of counterions or complexing ligands on the reduction potential. Bertilsson and Neujahr (*5*) have shown that the presence of complexing ions such as phosphate and thiols dramatically affects the rate of reaction between Hg(II) and methylcobalamin. A more complete study of the effect

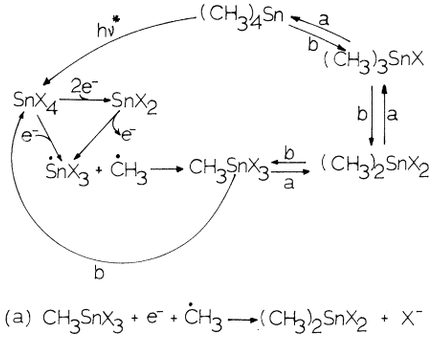


Fig. 4. Proposed biological cycle for tin. Counteranions are represented by *X*. The formation of a reactive radical species, ·SnX_3, may occur by single-electron oxidation of SnX_2 (*10*) or by single-electron reduction of SnX_4. (a) Homolytic cleavage of methylcobalamin by ·SnX_3 gives CH_3SnX_3, and successive reduction and alkylation of CH_3SnX_3 gives di-, tri-, and tetramethyl tin species. (b) Oxidative cleavage of alkyl tins by mixed function oxidases in a manner similar to that described for the mammalian microsomal system (*29*). (*) Alkyl tins are known to be degraded by photolysis ($h\nu$) to give free radicals as products (*16*).

of various anions on the methylation of Hg(II) by methylcobalamin has demonstrated a decrease in the rate constant for the demethylation reaction from 260×10^{-4} to $< 0.01 \times 10^{-4}$ sec^{-1} when the anion was changed from acetate to cyanide. The principal chemical species in solution when Hg(II) complexes with cyanide is $Hg(CN)_2$, but with acetate $HgOAc^+$ predominates since the second acetate dissociates readily. These workers were able to correlate the reactivity of Hg(II) with the stability constant of the Hg(II)-ligand complex. It would be interesting to examine the electrochemical properties of the various mercuric complexes to determine whether there are other correlations like this.

S-Adenosylmethionine–Dependent Reactions

In the late 1930's Challenger demonstrated that the bread mold *Scopulariopsis brevicaulis* was capable of synthesizing trimethylarsine from inorganic salts (*22*). Challenger's group demonstrated [^{14}C]methyl transfer from [^{14}C] methyl-labeled methionine to arsenic to give ^{14}C-labeled trimethylarsine as the product. Challenger concluded that the biomethylation of arsenic involved some "activated" methionine intermediate. The subsequent discovery of *S*-adenosylmethionine provides us with the biochemical basis for the synthesis of trimethylarsine. Clearly, methyl transfer to arsenic must occur by nucleophilic attack by some reduced arsenic salt on the C–S bond of *S*-adenosylmethionine. This would suggest that the biosynthesis of methylarsenic compounds occurs in a reducing environment. However, McBride and Wolfe (*23*) found that methylcobalamin was capable of functioning as methyl donor in the biosynthesis of dimethylarsine from arsenate or arsenite in cell extracts of a methane bacterium *Methanobacillus* M.O.H. It has been suggested that the Co–C bond of methylcobalamin is susceptible to nucleophilic attack by thiolate anions to give thioethers as products (*24*). If this is true, it seems reasonable to assume that reduced arsenic and selenium salts also function as nucleophiles.

It appears that methyl transfer reactions to the metalloids occur by either free radical attack on the Co–C bond of methylcobalamin or nucleophilic attack on *S*-adenosylmethionine as well as methylcobalamin. In either case, the reactions are likely to take place in anaerobic systems and will generate volatile products such as dimethylarsine, di-

methylselenide, or dimethylsulfide. These volatile products are slowly oxidized by molecular oxygen to give stable water-soluble species such as cacodylic acid. It is likely that cacodylic acid will represent the most abundant methylated arsenic compound in both freshwater and seawater. Methylarsenic compounds can be incorporated in phospholipids, as demonstrated by the isolation of a trimethylarsenic-containing phospholipid found as a natural product in fish, shellfish, and marine algae (*25*).

Conclusions

As we obtain more information on the movement of toxic elements in the biosphere, we will have a greater understanding of the environmental conditions required for the individual processes involved in biogeochemical cycling. In this article we have shown how oxidation-reduction conditions can be correlated with biomethylation processes. In recent years we have been able to establish biogeochemical cycles for mercury and arsenic (*3*). We can now formulate a biogeochemical cycle for tin in some detail (Fig. 4). The biomethylation of tin is interesting in two respects: (i) the use of tin by advanced industrial societies has more than doubled in the last 10 years (*26*); and (ii) the analytical methods to definitively establish whether methyl tin compounds accumulate in the food chain, including humans, have not yet been developed. Since methyl tin compounds are poisonous to the central nervous systems of higher organisms (*27*), we feel that it is critical to examine whether higher organisms provide a reservoir for some of the methyl tin compounds established as intermediates in the biogeochemical cycle for tin.

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30. Some of the research reported in this article was supported by grants from the National Science Foundation (PCM-17318) and the Public Health Service (AM 18101) and by the International Lead Zinc Research Organization.

Biological Nitrogen Fixation for Food and Fiber Production

What are some immediately feasible possibilities?

Harold J. Evans and Lynn E. Barber

Before the World War II period legumes were used extensively in the United States as a method of providing nitrogen to agricultural land. After this period, however, the supply of relatively inexpensive fertilizer nitrogen increased, values of land available for growth of legume cover crops rose, and interest in legumes and other biological nitrogen-fixing systems declined (*1*, *2*). It was recently estimated that commercial synthesis of fixed nitrogen in the United States required about 2.5 percent of our annual consumption of natural gas (*3*, *4*). The scarcity of appropriate sources of energy for the manufacture of nitrogen fertilizer and other problems associated with world food production have stimulated a reconsideration of recent agricultural practices (*2*, *5*). As a consequence, renewed interest in the possibility of increased dependence on nitrogen fixation has developed.

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Nitrogen-Fixing Systems

Some microorganisms that live in association with plants and others that exist under free-living conditions utilize solar energy stored as products of photosynthesis to biologically fix atmospheric nitrogen into compounds that may be used for the synthesis of protein and other products. Organisms that are capable of biologically fixing atmospheric nitrogen ordinarily require no other source, but those lacking this capacity are absolutely dependent on nitrogen from soil reserves or applied fertilizer. The best known of the nitrogen-fixing systems are the nodulated legumes. The beginning of our understanding of the symbiotic relationship between legumes and *Rhizobium* species was provided by Hellreigel and Wilfarth in 1888 (*6*), but inclusion of legumes in crop rotations as a method of supplying nitrogen was practiced in ancient agriculture (*7*). *Rhizobium* species with specificities for particular groups of legumes invade root hairs of plants such as alfalfa, clover, beans, and peas and induce root nodules that become packed with modified forms of the *Rhizobium* cells called bacteroids. If the legume cultivar and particular *Rhizobium* strain in