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Recent Studies on Biomethylation and Demethylation of Toxic Elements

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Methylcobalamin (methyl-B_{12}) has been implicated in the biomethylation of the heavy metals (mercury, tin, platinum, gold, and thallium) as well as the metalloids (arsenic, selenium, tellurium and sulfur). In addition, methylcobalamin has been shown to react with lead, but the lead-alkyl product is unstable in water.

Details of the kinetics and mechanisms for biomethylation of arsenic are presented, with special emphasis on synergistic reactions between metal and metalloids in different oxidation states. This study explains why synergistic, or antagonistic, processes can occur when one toxic element reacts in the presence of another.

The relative importance of biomethylation reactions involving methylcobalamin will be compared to those reactions where *S*-adenosylmethionine is involved.

Introduction

In the late 1930's Challenger (*1*) demonstrated that the bread mold *Scopuloriopsis brevicaulis* was capable of synthesizing trimethylarsine from inorganic arsenic salts. This pioneering research was elegant in two respects: it represented the first example of methyl-transfer to a toxic element, and it demonstrated ^{14}C methyl-transfer from ^{14}C methyl-labeled methionine to give us a classical example of how to use isotopes in experimental biology. In addition to testing arsenic, Challenger tested bread mold for the possible biomethylation of mercury but found no reaction (*2*). The lack of detection of methylmercury was probably related to the sparcity of good analytical techniques for methylmercury at that time. Challenger concluded that the biomethylation of arsenic involved some "activated" methionine intermediate. The discovery of "activated" methionine in the form of *S*-adenosylmethionine (SAM) 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 species, on the carbon-sulfur bond of SAM. Therefore, the biosynthesis of methylarsenic compounds must occur in a reducing environment. However, McBride and Wolfe (*3*) found that methyl-B_{12} was capable of functioning as methyl donor in the biosynthesis of dimethylarsine from arsenate or arsenite in cell extracts of *Methanobacillus* (M.O.H.). Since the Co–C σ bond has been shown to be susceptible to electrophilic attack (*4*), nucleophilic attack (*5*), and homolytic attack by free-radical species (*6*), it is important to determine how methyl-groups are transferred from methyl-B_{12} to arsenic. Schrauzer et al. (*7*) have formulated a mechanism for B_{12}-dependent synthesis of dimethylarsine which involves inorganic arsenic salts functioning as electrophiles. In this communication we review the alternative mechanisms for the biosynthesis of methyl-arsenic compounds with a view to providing a chemical basis for determining which methylated species of arsenic are likely to reach significant concentrations in aqueous systems.

Results and Discussion

Jernelov (*8*) presents a comprehensive set of environmental characteristics and properties that, when determined, permit an evaluation of potential undesirable inputs of a metal: (1) production and

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emission in relation to natural flux; (2) residence times in various reservoirs; (3) bioaccumulation, both passive and active; (4) physical and chemical properties relating to dispersion (e.g., volatility, adsorption tendencies, dissociation–association reactions, formation of insoluble precipitates); (5) toxicity to aquatic organisms; (6) toxicity to man and other mammals; (7) long-term biological effects on ecosystem metabolism; and (8) transformation reactions by organisms. Jernelov's monograph examines selected toxic elements in detail.

Table 1 classifies elements in terms of their relative toxicity and availability (*9*, *10*). Biologists should give careful consideration to those metals categorized as very toxic and relatively accessible. Toxic but insoluble but rare metals deserve notice as well, because insoluble toxic elements can be rendered soluble by man-made chelating agents such as polyphosphates and nitrilotriacetic acid (NTA), both of which are added to our water supply via detergents. For example, the very toxic lanthanides will complex with polyphosphate or NTA.

Table 1. Classification of elements according to their toxicity.[a]

Noncritical	Very toxic and relatively accessible	Toxic but very insoluble or very rare
Na	Be	Ti
K	Co	Hf
Mg	Ni	Zr
Ca	Cu	W
H	Zn	Nb
O	Sn[b]	Ta
N	As[b]	Re
C	Se[b]	Ga
P	Te[b]	La[c]
Fe	Pd[b]	Os
S	Ag	Rh
Cl	Cd	Ir
Br	Pt[b]	Ru
F[d]	Au[b]	Ba
Li	Hg[b]	
Rb	Tl[b]	
Sr	Pb[b]	
Al	Sb	
Si	Bi	

[a]Elements omitted from this table should not be neglected in the environmental sense. For example, iodine and manganese are important elements, but they fit more than one category for the above classification.

[b]Metals having metal alkyls which are stable in aqueous systems which have been reported to be biomethylated.

[c]All the lanthanides are very insoluble and some are very rare.

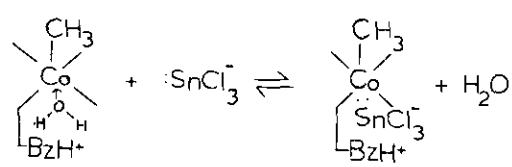
[d]Some may argue with this designation, but we do add fluoride to drinking water.

Toxic metals that form organometallic compounds, especially the metalalkyls (e.g., methylmercury), deserve special concern. Most metal alkyls are poisonous to the central nervous systems of higher organisms, and these compounds do accumulate in cells. Metal alkyls that are stable in water and can be synthesized include the following toxic elements: Hg, Sn, As, Se, Te, Pd, Pt, Au, Tl, and Pb.

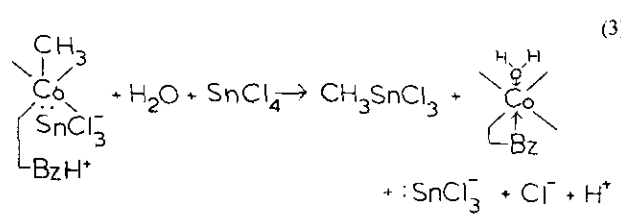
The general survey for the biomethylation of toxic elements presented above fails to deal with the problem of synergistic or antagonistic reactions which can occur in the presence of mixture of elements. Furthermore, the survey fails to deal with the importance of "oxidation state" of that element which is being biomethylated. A good example of the latter is presented in some of our studies with tin (*11*).

Recently we have shown that stannic salts do not react with methyl-cobalamin directly, but stannous salts react slowly to give a methyl–stannic complex as the product. At first glance the reaction appeared to progress by oxidative addition. Schrauzer and Kratel (*12*) have prepared alkyl-tin–cobalt derivatives for cobaloximes. Patmore and Graham (*13*) have demonstrated tin insertion into the cobalt–carbon bond for alkyl–cobalt tetracarbonyl complexes, and so insertion into the Co–C bond of methyl-B_{12} seemed reasonable. However, a detailed study of the reaction between stannous chloride and methylcobalamin suggests that the role of stannous ion is to facilitate electrophilic attack by contaminating stannic ion. We have shown that the reaction rate is dependent on the concentration of Cl^-, indicating the $SnCl_3^-$ may have a labilizing effect by coordinating *trans* to the Co–C bond [Eqs. (1)-(3)]. The initial reaction probably involves

$$:SnCl_2 + Cl^- \rightleftharpoons :SnCl_3^- \qquad (1)$$



(2)



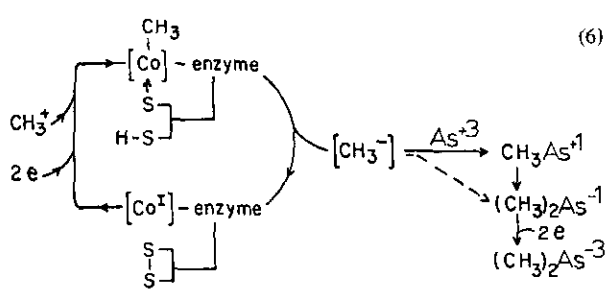
(3)

the reaction of chloride ion with stannous chloride to give the weak nucleophile $SnCl_3^-$, which coordinates to the cobalt atom to facilitate electrophilic attach by stannic chloride to give CH_3SnCl_3 as the product. Chloride ion is critical to this reaction because it establishes a reasonable concentration of $SnCl_3^-$.*

From this survey it appears that heterolytic cleavage of the Co–C bond is a general reaction for both strong and weak electrophiles. However, weak electrophiles may need assistance by the coordination of good electron donors transaxial to the methyl group. Since metalloids are more nucleophilic in character from electrophilic, then methyl-B_{12} would need the Co–C bond labilizing by a very strong base to facilitate electrophilic attack by metalloids of higher oxidation state.

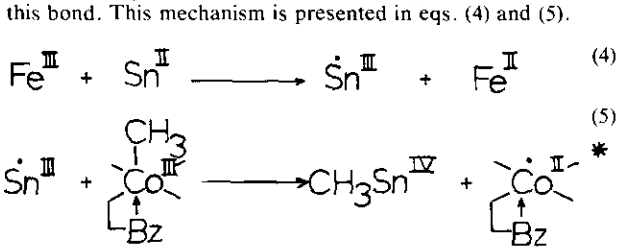
Reactions with Metalloids

Schrauzer has postulated that the coordination of thiols to methyl-cobalamin facilitates heterolytic cleavage of the Co–C bond by the following electrophiles: (1) H^+ to give methane, (2) carbon dioxide to give acetic acid, (3) arsenic salts to give alkylarsenic compounds, and (4) selenium salts to give alkyl selenium compounds (*7*, *14*). Schrauzer predicts that the coordination of the thiol increases the electron density on the methyl group to generate a "krypto" carbanion which is readily displaced by arsenic salts and selenium salts [Eq. (6)].



(6)

*Since this manscript was written, we have shown that tin is capable of homolytic cleavage of the Co-C σ bond. Recent experiments implicate Sn^{III} as the chemical species which cleaves this bond. This mechanism is presented in eqs. (4) and (5).



(4)

(5)

Table 2. Coupling constants, J $^{13}C-^1H$ of [^{13}C]methylcobalamin samples.

Sample	Solvent	J $^{13}C-^2H$, Hz	A, Hz	B, Hz	A-B, Hz
[^{13}C]Methyl-cobalamin	0.1M KH_2PO_4				
	Ph 7.0	138	69	69	0
	pH 4.0	138	68	70	2
	pH 1.5	146	73	73	0
[^{13}C]Methyl-cobalamin-glutathione	0.1M KH_2PO_4, pH4.0	138	69	69	0

Table 3. Chemical shifts by ^{13}C NMR.

Sample	Concn		Chemical shift (ppm)[a]
[^{13}C]Methylcobalamin	8.6mM	pH 5.6	+7.55
	30 mM	pH 2.8	+4.65
	34 mM	pH 1.1	+0.02
[^{13}C]Methylcobalamin (34 mM)			
Glutathione	0.4 M	pH 3.6	+6.16
Glutathione	0.8 M	PH 3.6	+6.27
Glutathione	0.8 M	pH 2.8	+5.03
[^{13}C]Methylcobinamide	30 mM	pH 7.0	+0.36
	15mM	pH 7.0, T=20°C	−0.14
[^{13}C]Methylcobinamide glutathione	30 mM 0.5M	pH 7.0	+0.30

[a]Chemical shift given relative to TMS at 0.00 ppm. Positive sign indicates shift to low field. All studies taken at 40°C unless otherwise noted.

Using this scheme, Schrauzer suggests mechanisms for the cobalamin-dependent biosynthesis of methane, acetic acid, dimethylarsine, and dimethyl-selenide. Although this mechanism is very appealing, it is important to determine whether thiols coordinate to methylcobalamin. Several laboratories have reported on the interaction of thiols to both cobalamins and cobaloximes (*5*, *15*). In fact, we reported that reduced gluthathione is capable of displacing benzimidazole and coordinating to the cobalt atom for the important B_{12}-coenzymes methylcobalamin and 5′-deoxyadenoxylcobalamin (*15*). Our conclusions were based on ultraviolet-visible titrations and the pH dependence for the formation of reduced glutathione complex. We have re-examined this reaction using ^{13}C and ^1H NMR and we can find no evidence for the formation of a stable coordination complex between these corrinoids under conditions where we have (*6*) a 25-fold excess of glutathione over cobalamin (Tables 2 and 3). This does not rule out the possibility that a cysteinyl or methionyl residue of an enzyme might form a complex with bound methylcobalamin and activate the Co–C bond for electrophilic attack by arsenic salts. It

seems much more likely however that biomethylation of arsenic by either SAM or methyl-B_{12} occurs by nucleophilic attack by arsenic salts of lower oxidation state. This would indicate that the biosynthesis of methylarsenic compounds would be favored in a strictly anaerobic environment, where only the volatile methylarsines would be released into the aerobic environment.

Conclusions

In this communication we take all the available experimental data on the biomethylation of arsenic and we believe that the biomethylation of arsenic by methyl-B_{12} occurs in anaerobic ecosystems. The volatile species arsine, demethylarsine, and trimethylarsine, will be released into the aerobic environment at the sediment/water, water/air, or soil/air interface. These alkylated arsenic compounds are slowly oxidized in air and water to give steady state concentrations of methylated arsenic compounds of higher oxidation state. It is likely that cacodylic acid will represent the most abundant methylated arsenic compound present in both freshwater and sea water. Cacodylic acid should exist in a steady state concentration due to oxidative demethylation by aerobic bacteria. In addition methylarsenic compounds may be incorporated by biosynthesis into phospholipids which are found in most marine organisms (*16*).

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