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Methyl transfer from a hydrophobic vitamin B_{12} derivative to arsenic trioxide

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ABSTRACT

The methylation reaction of arsenic trioxide conducted at 37 °C and pH 7.0 for 24 h using hydrophobic methylated vitamin B_{12}, (methyl) (aquo) heptamethylcobyrinate perchlorate, CH_3B_{12} ester, as a methyl donor in the presence of reduced glutathione (GSH) yielded monomethylarsonous acid (MMA), dimethylarsinic acid (DMA), and trimethylarsine oxide (TMAO) as products with a methylation rate over 95%. In contrast, when methylcobalamin (CH_3B_{12}) was used as the methyl donor, only MMA and DMA were produced and the methylation rate dropped to around 20%. Reductive demethylation of a methyl-corrinoid coordination complex mediated by GSH is suggested as a mechanism of methyl transfer to arsenic trioxide. The differences observed for different corrinoid coordination complexes with respect to the reactivity of methyl transfer to arsenic is ascribable to differences inherent in the base-on (CH_3B_{12}) and base-off (CH_3B_{12} ester) natures of the compounds.

1. Introduction

In general, organometallic compounds are more toxic than the inorganic metal compounds from which they derive. Mercury, lead and tin obey this general rule, however, arsenic is an exception [1]. The toxicity of arsenic compounds is markedly dependent on their chemical structure, and some methylated arsenic compounds have lower toxicities than those of inorganic arsenic compounds. In particular, arsenobetaine [AB;$Me_3As^+CH_2CO_2^-$; (trimethyl arsonio) acetate] has a low toxicity (LD_{50}, 10 g/kg, mice oral) and is found in high levels in fishery products [2]. The acute toxicity of AB, as determined from animal experiments, is approximately one three-hundredth of that of arsenic trioxide [iAs(III); Arsenite; As_2O_3; LD_{50}, 0.03 g/kg, mice oral] [2]. Moreover, as a result of studies on the metabolism and excretion of AB in human and animals, it is now widely accepted that AB is chemically stable, has a low tissue affinity, and is rapidly excreted from the human body [3]. Although AB is produced by the action of marine organisms, there have been no successful attempts to artificially synthesize AB in a safe and cost-effective way. Recently, we have successfully demonstrated a new two-step synthetic pathway developed for the transformation of arsenic trioxide into AB. It involves treatment of iAs(III) with methylcobalamin or methylcobyric acid in the presence of glutathione (GSH) to give trimethylarsine oxide (TMAO) with a high selectivity and a high conversion rate followed by subsequent treatment of TMAO with iodoacetic acid in the presence of GSH to give arsenobetaine with a high yield [4].

Trimethylarsine oxide (TMAO) is a compound with low toxicity (LD50, 10.6 g/kg, mice oral) [5] and is an important intermediate in AB synthesis. The reaction mechanism of the one-step synthesis of TMAO from arsenic trioxide using vitamin B_{12} derivatives has attracted much attention. Further improvements in the efficiency and performance is expected through further elucidation of the reaction mechanism. Hydrophobic methylated vitamin B_{12}, (methyl)(aquo) heptamethylcobyrinate perchlorate, CH_3B_{12} ester, is prepared by hydrolyzing and methylesterification of the seven amido groups present in the periphery of cobalamin [6]. The presence of seven methyl groups within the corrin ring makes the compound hydrophobic (Supplementary material) [6]. Due to the absence of a dimethylbenzimidazole group coordinated to the cobalt atom in the α plane of the corrin ring, the compound is always present in a solution in a base-off form. In contrast, methylcobalamin, a natural derivative of vitamin B_{12}, is present in a base-on form because a dimethylbenzimidazole group is coordinated to the cobalt when it is in neutral water solution [7]. The oxidation–reduction potential of a base-off type corrinoid coordination complex is shifted to a more positive value than that of base-on type compound [8]. Thus, base-off CH_3B_{12} ester has been shown to be more easily reduced compared to base-on methylcobalamin. The base-off corrinoid coordination complex (CH_3B_{12} ester) is so structured that the central metal cobalt atom is elevated from the plane of the corrin ring. Thus, access of reactive species to the Co–C bond becomes easier, and reaction more likely. From a practical point of view it is expected that CH_3B_{12} ester can be used

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repeatedly as a methyl donor in aqueous solution by extracting it with organic solvent and remethylating the compound used in the reaction (Fig. 1).

From such a point of view, the methyl transfer reaction from hydrophobic methylated vitamin B_{12} derivative to arsenic trioxide was examined in comparison with the reactivity of natural B_{12} (methylcobalamin) and our knowledge of the reaction mechanism improved.

2. Experimental

2.1. General procedure for arsenic methylation

Ultrapure 18 MΩ deionized water, DIW (Millipore, Japan) was used in the preparation of all reagents and standards. Arsenic trioxide [iAs(III)], arsenic pentaoxide [iAs(V)], monomethylarsonous acid (MMA), dimethylarsinic acid (DMA), trimethylarsine oxide (TMAO), tetramethylarsonium iodide (TeMA) were purchased from TRI Chem (Yamanashi, Japan). (Methyl) (aquo) heptamethylcobyrinate perchlorate, CH_3B_{12}ester, was synthesized as previously described [6] (Supplementary material). Methylcobalamin (CH_3B_{12}) was obtained from Wako Chemical (Tokyo, Japan). Glutathione (GSH, γ-Glu-Cys-Gly; reduced form) was purchased from Sigma. The reaction mixture (500 μL), containing 30 μmol/L of iAs^{III}, 22 mmol/L of GSH in 100 mmol/L of Tris–HCl buffer and 150 μmol/L of CH_3B_{12} was incubated at 37 °C for several hours. When CH_3B_{12}ester was used as a methyl donor, a methanol stock solution of CH_3B_{12}ester (17 mM) was prepared. The reaction mixture (500 μL including 1% methanol), containing 30 μmol/L of iAs(III), 22 mmol/L of GSH in 100 mmol/L of Tris–HCl buffer and 150 μmol/L of CH_3B_{12}ester was incubated at 37 °C for several hours. The reaction mixtures sampled (20 μl) with/without H_2O_2 treatment were analyzed by HPLC (Agilent 1100) /ICP-MS (Agilent 7500ce) on a cation exchange column (Shodex RSpak NN-414 (150 mm × 4.6 mm i.d., Tokyo, Japan) at a flow rate of 0.4 ml/min of mobile phase (5 mM nitric acid/6 mM ammonium nitrate/ 1.5 mM pyridine dicarboxcylic acid) at 40 °C. The eluent was directly introduced into the ICP-MS, and the arsenic signal (*m/z* 75) was monitored every second. During the reaction of GSH, iAs(III) and CH_3B_{12} or CH_3B_{12}ester, samples of the reaction mixture were taken at various time intervals, diluted 2- to 10-fold, and analyzed immediately using HPLC–ICP-MS with a cation exchange column for separation of iAs(III), iAs(V), MMA(V), DMA(V), TMAO and TeMA. For the chromatograms shown in Fig. 2, the starting reaction

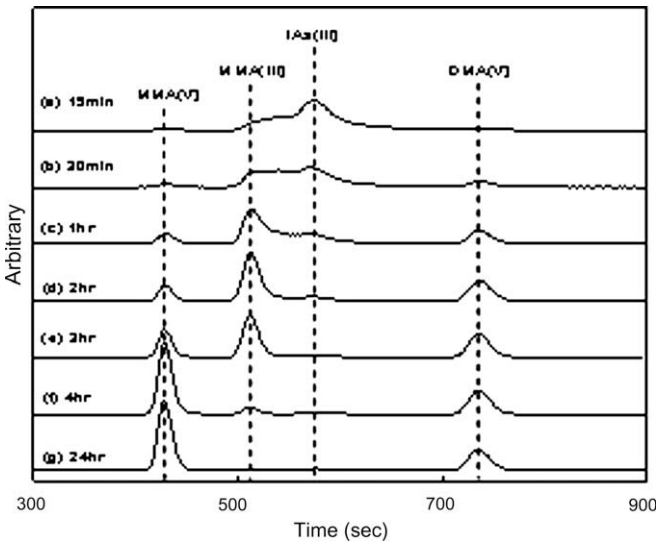

Fig. 2. HPLC–ICP-MS analysis of methylation of iAs(III) with CH_3B_{12} ester. 30 μM iAs(III) with GSH (22 mM), and CH_3B_{12} ester (150 μM) in 100 mM Tris–HCl buffer at 37 °C and pH 7 for various reaction times.

mixture was comprised of 30 μmol/L iAs(III) in 100 mmol/L Tris–HCl buffer with 150 μmol/L CH_3B_{12}ester, and 22 mmol/L GSH.

For calculation of the methylation rate, treatment with hydrogen peroxide was conducted prior to sampling from reaction solution and prior to analysis with HPLC–ICP-MS. As will be described later, the purpose of the procedure is to oxidize monomethylarsonous acid, MMA(III), for which no standard sample is available, to monomethylarsonic acid, MMA(V), which does have a standard sample available. In this way, it is possible to quantitate MMA(III) through the analysis of MMA(V). Methylation efficiency was defined according to the following equation:

$$100\%[\mathrm{DMA(V)} + \mathrm{MMA(V)} + \mathrm{TMAO}]/[\mathrm{iAs(III)} + \mathrm{iAs(V)} + \mathrm{DMA(V)} + \mathrm{MMA(V)} + \mathrm{TMAO}]$$

2.2. Electronic spectrum measurement

Electronic spectra were measured by a ultraviolet-visible spectrophotometer (Hitachi High Technologies U-2910 Double Beam

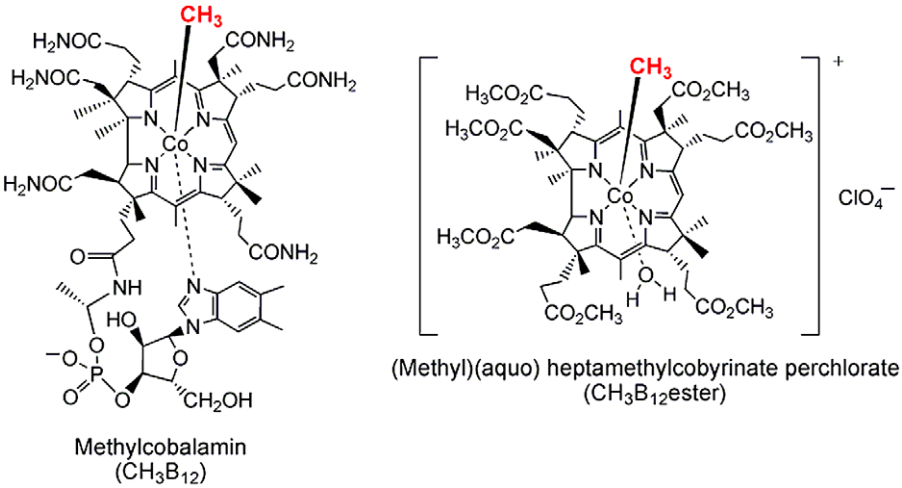

Fig. 1. Structure of vitamin B_{12} derivatives.

Spectrophotometer). Samples were measured in 1-cm quartz cells at room temperature, in the dark.

3. Results and discussion

3.1. Identification of reaction products by using HPLC-ICP-MS

After mixing CH_3B_{12}ester (150 μM), reduced glutathione (GSH - 20 mM) and arsenic trioxide (iAs(III) – 20 μM), samples were taken at designated times. Immediately after a 10-fold dilution of the samples, the progress of the methylation reaction of arsenic trioxide was analyzed by HPLC–ICP-MS. Fig. 2 shows a representative chromatogram. In addition to the starting material, iAs(III), 4 additional peaks were detected. By comparing the retention times of the peaks with those of commercial samples of iAs(V), MMA(V), dimethylarsinic acid [DMA(V)], trimethylarsine oxide (TMAO), and tetramethylarsonium (TeMA) as standard arsenic compounds, the presence of MMA(V), DMA(V) and TMAO was successfully identified. With this method it was not possible to identify a peak with a retention time of 510 s that appeared between the peaks of MMA(V) and iAs(III). To identify this peak, therefore, solutions sampled from reaction mixtures were treated with 20 μL of a 30% aqueous solution of hydrogen peroxide and analyzed with HPLC–ICP-MS. The peak with retention time of 510 s disappeared and the MMA(V) peak increased instead. This 510 s peak can reasonably be assigned to MMA (III), as suggested previously by Heitkemper et al. [9]. When conducting the reaction with GSH, Se(IV), and iAs(III), they found a similar disappearance of this peak, with a concomitant rise in the MMA(V) peak, after treatment with aqueous solution of hydrogen peroxide and ascribed it to MMA(III).

Heitkemper et al. [9] also observed the production of an arsenic containing complex at an early stage of the methylation reaction performed using methylcobalamin, GSH and iAs(III). Analysis of early samples with HPLC–ICP-MS showed a pronounced tailing of the iAs(III) peak. Addition of aqueous solution of hydrogen peroxide to this sample solution resulted in the disappearance of the iAs(III) peak, and its tail, and an increase in the iAs(V) and MMA(V) peaks. They pointed out that the tailing could be ascribed to GSH–MMA(III) and/or GSH–iAs(III). In experiments using methylcobalamin conducted by the present authors, marked tailing was observed for peaks ascribed to iAs(III) and MMA(III) in chromatogram taken at early times. Similar tailing was also observed for peaks ascribed to iAs(III) and MMA(III) when CH_3B_{12}ester was used (Fig. 2). These findings suggest the production of a GSH–MMA(III) and/or GSH–iAs(III) complex also takes place when CH_3B_{12}ester was used as a methyl donor.

3.2. Reaction kinetics

Kinetic profiles for the reaction of CH_3B_{12}ester, GSH, and iAs(III) are shown in Fig. 3. They show that the concentrations reached steady states after 4–5 h. The starting material, iAs(III) decreased greatly over a period of 2 h. The peak ascribed to MMA(III) increased rapidly at the start of the reaction, reaching saturation at 2–3 h, and then decreased. In keeping with the decrease in MMA(III) during this later time period, the MMA(V) peak increased. Although there was no change in the DMA after 5 h, the amount of MMA(III) decreased and that of MMA(V) increased. Observations 5 h and thereafter seem to indicate that MMA(III) was not methylated further and was oxidized to MMA(V), instead of changing to DMA.

The relative proportions of methylated arsenic concentrations after 24 h of incubation and H_2O_2 treatment (Fig. 2) are 68.7% MMA(V), 27.2% DMA(V) and 0.1% TMAO (Table 1). It is quite unusual that, in this experiment, more than 95% of the starting arsenic trioxide was converted into MMA(III), MMA(V), DMA and TMAO, since the peak methylation efficiency using CH_3B_{12} and GSH was only 20% (Table 1). We have also found that the methylation efficiency of CH_3B_{12} and GSH was promoted by visible light irradiation (unpublished data).

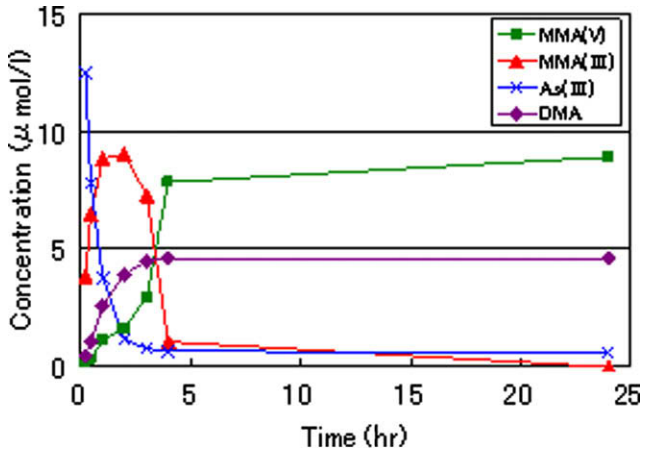

Fig. 3. Kinetic profiles of CH_3B_{12} ester methylation. 30 μM As(III) with GSH (22 mM), and CH_3B_{12} ester (150 μM) in 70 mM Tris–HCl buffer incubated at 37 °C and pH 7 for 0–24 h incubation before H_2O_2 treatment.

Table 1
Arsenic methylation mediated by methyl-corrins (CH_3B_{12} and CH_3B_{12} ester).

Entry	Methyl donor	Absolute yield (%)[a]				
		iAs[b]	MMA	DMA	TMAO	TeMA
1	CH_3B_{12} ester	0	67.8	27.2	0.1	0
2	CH_3B_{12}	77.0	17.2	2.8	0	0

[a] Methylation reactions were carried out in Tris–HCl buffer (pH 7) at 37 °C for 24 h. Initial concentrations were as follows: CH_3B_{12} (methylcobalamin) or CH_3B_{12} ester, (methyl)(aquo)heptamethlycobyrinate perchlorate: 1.5 × 10–4 M, GSH: 2.2 × 10–2 M, iAs: 3.0 × 10–5 M.
[b] Products were assayed by HPLC–ICP-MS. iAs includes arsenite [iAs(III)] and arsenate [iAs(V)].

3.3. Spectroscopic studies

Changes produced in the ultraviolet-visible absorption spectrum of methylcobalamin by the addition of GSH (at room temperature and pH 5) in the absence of iAs(III) were followed. Before the addition of GSH, the absorption spectrum typical of base-on methylcobalamin was observed (Fig. 4A). In the spectrum obtained 30 min after reaction induced by the addition of GSH, the corrinoid αβ absorption band (490–540 nm) shifted to a longer-wavelength region of 520–570 nm (Fig. 4B), suggesting the coordination of GSH or GS-ion, compounds with strong electron-donating properties, to the cobalt atom. With a further lengthening of the reaction time, an absorption band ascribable to Co(II) became clearly visible at 465 nm, indicating that methylcobalamin reacted with GSH in the absence of iAs(III) and that reduction of CH_3B_{12} by GSH was occurring. On the other hand, when CH_3B_{12}ester was used as a methyl donor in place of CH_3B_{12}, the corrinoid αβ absorption band (450–540 nm) shifted to 470 nm, a band ascribable to Co(II), within 5 min after the addition of GSH (Fig. 6 in Supplementary material). This indicates that CH_3B_{12}ester reacted with GSH in the absence of iAs(III) and that reaction of CH_3B_{12}ester with GSH is much faster than that of CH_3B_{12}.

In a previous report, it was suggested that methylcobalamin produced either a Co(I) species plus a methyl radical or a Co(II)

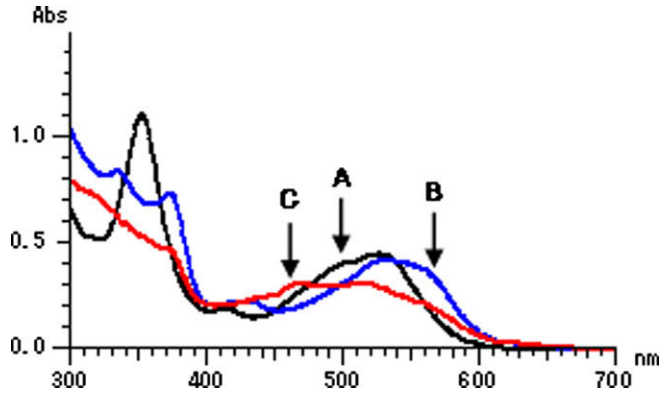

Fig. 4. UV–visible absorption spectra of CH_3B_{12}. GSH (20 mM), and CH_3B_{12} (64 μM) in 100 mM citrate buffer were incubated at 37 °C and pH 5.0 for various time intervals. Black line (A): before GSH added, Blue (B): after 30 min, Red (C): after 12 h. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

species plus a carbanion species through reductive demethylation brought about electrochemically in a one-electron reduction reaction (Formula 1) [10]

$$CH_3 - Co(III) + e^- \rightarrow CH_3^{\cdot} + Co(I) \quad \text{or} \quad CH_3^- + Co(II) \tag{1}$$

It is known that Co(I) species change in an aerobic aqueous solution to aquocobalamin through oxidation (Formula 2) [11]. Aquocobalamin is in equilibrium with hydroxocobalamin (Formula 3) in aqueous solution and produces glutathionylcobalmin through reaction with GSH (Formula 4) [11–12]

$$Co(I) + H_2O + O_2 \rightarrow Co(III) - OH_2 \tag{2}$$

$$Co(III)OH + H^+ \rightarrow Co(III)OH_2 \tag{3}$$

$$Co(III)OH + GSH \rightarrow Co(III)SG + H_2O \tag{4}$$

As regards reactions between cobalamins and thiols, it is known that cobalamins (methylcobalamin, adenosylcobalamin, cyanocobalamin, hydroxocobalamin) can oxidize cysteine catalytically in an aerobic aqueous solution [11]. As the reaction investigated in the present study was conducted an aerobic aqueous solution, it is possible that methylcobalamin oxidized GSH catalytically (Formula 5). Or, to put it in another way, GSH reductively activated methylcobalmin (Formula 1)

$$2GSH \rightarrow GS - SG + 2H^+ + 2e^- \tag{5}$$

Spectroscopic studies (Fig. 4) suggest that CH_3B_{12} reacts with GSH, producing Co-glutathione complex (Formula 6–7) followed by the reductive demethylation of the CH_3–Co(III) complex to a Co(II) species and a methyl radical. It is plausible that this demethylation is accelerated by a trans axial -effect of coordination by GS(H) (Formula 8). The reaction of CH_3B_{12}ester with GSH is faster than that of CH_3B_{12} (Fig. 6 in Supplementary material), suggesting that base-off methyl-corrinoids are more susceptible to coordination by GS(H) and to reductive demethylation than is base-on CH_3B_{12}

$$CH_3 - Co(III) + GSH \rightarrow CH_3 - Co(III) - SHG \tag{6}$$

$$CH_3 - Co(III) - SHG \rightarrow CH_3 - Co(III) - SG + H^+ \tag{7}$$

$$CH_3 - Co(III) - SG \rightarrow CH_3^{\cdot} + Co(II) - SG \tag{8}$$

3.4. Mechanism

When methylcobalamin transfers a methyl group non-enzymatically from cobalt to a heavy metal ion, cleavage of the Co–C bond is necessary. Depending on the conditions, the cleavage of this bond yields a carbonium ion (CH_3^+), a radical ($CH_3^{\cdot}$), or a carbanion (CH_3^-). Transfer to a mercury ion (Hg^{2+}), lead (Pb^{2+}), or palladium (Pd^{2+}) by cobalamine are examples of reactions ascribable to electrophilic attack on a carbanion by positively charged heavy metal ions. Considering that arsenic trioxide in aqueous solution is considered to be an analog of the phosphate anion, it is easily understood that the electrical nature of this compound differs greatly from that of a heavy metal ion. Thus, it is hard to imagine that the arsenic reaction proceeds via a carbanion.

A possible alternative mechanism for the biological methylation of arsenic, involving the reduction of As(V) to As (III) and followed by an oxidative methylation reaction[13]. It is generally accepted that *S*-adenosylmethionine (SAM) functions as a methyl group donor in this reaction [14]. The methyl group of SAM dissociates as a carbonium ion (CH_3^+), which then methylates iAs(III) oxidatively. As a consequence, MMA(V) [O=As(V)$CH_3(OH)_2$] is produced as the primary product. If methyl transfer from methyl-corrins proceed in a similar way in the case of SAM, MMA(V) should be obtained as a primary product from CH_3-Co(III) and iAs(III), even in the absence of GSH. However, methylation of iAs(III) does not proceed in the absence of GSH, and kinetic profiles indicated that MMA(III) [As(III)$CH_3(OH)_2$] was oxidized to MMA(V) in the reaction time period (Fig. 3), suggesting that methyl transfer to arsenic trioxide by CH_3B_{12}ester does not proceed via oxidative methylation involving a carbonium ion species (CH_3^+).

Next, we would like to consider a radical mechanism. There are several reports that concern the photochemistry of methylcobalamin [15–17]. In this case, the overall reaction can be expressed by Formula 9, while the elementary reactions are represented by Formulas 10–13. The Co–C bond undergoes homolytic cleavage to yield Co(II) and a methyl radical (Formula 10). To confirm whether arsenic trioxide functions as radical trap, mixed solution of iAs(III) and methylcobalamin was irradiated with light. Production of MMA(V) was confirmed in the absence of GSH, indicating that arsenic trioxide does function as a methyl radical trap (Formula 11) [18]

$$CH_3 - Co(III) + As(III)(OH)_3 + H^+ \xrightarrow{h\nu} Co(III)OH_2 + As(III)(CH_3)(OH)_2 \tag{9}$$

$$CH_3 - Co(III) \xrightarrow{h\nu} Co(II) + CH_3^{\cdot} \tag{10}$$

$$As(III)(OH)_3 + CH_3^{\cdot} \rightarrow As(III)(CH_3)(OH)_2 + OH^{\cdot} \tag{11}$$

$$Co(II) + OH^{\cdot} \rightarrow Co(III)OH \tag{12}$$

$$Co(III)OH + H^+ \rightarrow Co(III)OH_2 \tag{13}$$

The next question concerns whether a methyl radical can be produced from CH_3B_{12} without using light irradiation. Lexa et al. demonstrated the production of Co(I) and methyl radical via an electrochemical one-electron reduction of methylcobalamin (Formula 1) [10]. In addition, as discussed earlier, cobalamins catalytically promote an oxidative reaction of thiols [11]. Thus, GSH which possesses the sulfhydryl group, can reduce CH_3B_{12}. It is possible to explain methyl transfer from CH_3B_{12} to iAs(III) by a series of reactions that follow Formulas 10–11, i.e. the production of a methyl radical through reductive demethylation of CH_3B_{12} to iAs(III) (Formula 1), and the consequent production of MMA(III).

Next we would like to consider the activation of arsenic by GSH. As has been suggested previously, iAs(III) is methylated nonenzymatically in the presence of methylcobalamin and GSH by means of nucleophilic attack of the As–GSH complex on cobalt [9,19]. It has also been reported that iAs(III) is enzymatically methylated by arsenic methyltransferase (Cyt19) and *S*-adenosyl-L-methionine (SAM) in the presence of GSH through nucleophilic attack of a lone pair from a thiol group of an arsenic-GSH complex on the cationic sulfur of SAM, followed by SAM methyl group transfer to the arsenic–GSH complexes [22]. No further methylation yielding trimethylarsine or its oxide was observed either in the case of

methylcobalamine or Cyt19 plus SAM. The production of glutathione conjugates from GSH and iAs(III) has been reported (Formulas 14–15). As described previously, chromatographic analysis suggested the production of a complex between iAs(III) and GSH, as well as one between MMA(III) and GSH, in our system. It is possible that these compounds, which have the structure of As(III)–GSH and As(III)–SG complexes, are activating arsenic trioxide or methylated arsenic towards attack by a methyl radical

$$As(OH)_3 + GSH \rightarrow As(SG)(OH)_2 + H_2O \quad (14)$$

$$As(SG)(OH)_2 + GSH \rightarrow As(SG)_2(OH) + H_2O \quad (15)$$

$$As(SG)_2(OH)_2 + GSH \rightarrow As(SG)_3 + H_2O \quad (16)$$

The mechanism proposed by Zakharian and Aposhian [19] and Heitkremper et al. [9] for the mechanism of non-enzymatic methylation of arsenic trioxide by methylcobalamin is that methylation occurs by nucleophilic attack on Co–C bond of an arsenite–GSH complex. As support for this explanation, the evidence of methylation of thiol by methylcobalamin reported by Hogenkamp et al. is cited as an example demonstrating the progress of heterolytic cleavage of the Co–C bond of methylcobalamin by the nucleophilic thiolate anion under relatively mild conditions [20–21]. However, according to the explanation by Hogenkamp et al., the attack by thiolate anion progressed only under alkaline and severely controlled anaerobic conditions [20–21]. In contrast, as we have already explained, corrinoids can perform catalytic oxidative reactions with thiols under aerobic conditions [11]. Thus, it may be taken that GSH can activate methyl-corrinoids in a reductive fashion under aerobic conditions (Formulas 1–5). Our absorption spectral data support this idea. In the absence of iAs(III) the reaction between GSH and methylcobalamin under aerobic conditions resulted in a shift of absorption band to the red, indicating the occurrence of a mutual reaction between GSH and the Co complex. In addition, an absorption band ascribed to Co(II) species was observed (Fig. 4), suggesting the sequence of reactions described in Formulas 1–5.

The rate of methylation (>95%) of arsenic trioxide by CH_3B_{12} ester in the presence of GSH was markedly higher than the rate using methylcobalamin (~20%). We offer two possible explanations for this difference. First, the oxidation–reduction potential of the base-off corrinoid complex is shifted to a more positive value than that of the base-on complex. Thus, the base-off compound is more liable to undergo reduction. As CH_3B_{12} ester is present in a base-off form, it is conceivable that it is more liable to undergo reduction than CH_3B_{12}, which is present in a base-on form under near-neutral conditions. Second, CH_3B_{12} ester (base-off) is more susceptible to the coordination of GS(H) to cobalt than that of CH_3B_{12} (base-on). Trans axial-effects by the coordination of GS(H) to cobalt may promote the reductive demethylation of methyl-corrinoids, producing (CH_3^-) and Co(II) species. Therefore, for the reductive activation of Co–CH_3 by GSH as shown in Formulas 1–5, CH_3B_{12} ester is more likely to react than methylcobalamin. Jacobson et al reported on the speed of catalytic oxidative reaction of 2-mercaptoethanol by derivatives of vitamin B_{12} [11]. They found that the base-off compounds (cobinamids) were more than 2 orders of faster than base-on compounds (cobalamins).

Regarding the coordination complex, from a stereochemical point of view, we offer the following explanation. In a base-on coordination complex the central cobalt atom is located in the plane of the corrin ligand, while in a base-off coordination complex, the cobalt atom is elevated above the plane of corrin and lies in the β plane. Thus, the Co–CH_3 bond present in base-off CH_3B_{12} ester is more liable to undergo attack by reaction intermediates than that in base-on methylcobalamin. Thus, the fact that the methyl radical transfer reactivity of CH_3B_{12}ester to arsenic trioxide is higher than that of methylcobalamin can be explained from the viewpoint of oxidation–reduction potential and stereochemistry.

4. Conclusion

This is the first report that has shown the process of methyl transfer from methylated hydrophobic vitamin B_{12}, CH_3B_{12}ester, in the presence of GSH. Under mild conditions (aqueous solution, 37 °C, pH 7) arsenic trioxide was methylated with over 95% efficiency. In contrast, CH_3B_{12} was only 20% methylated, similar to the maximum methylation value found in previous reports [9]. The reaction mechanism of the methyl radical transfer to arsenic was explained by cleavage of the Co–C bond through reductive activation of GSH and subsequent attack on the arsenic trioxide by the generated methyl radical. GSH forms a coordination complex with arsenic and makes arsenic more susceptible to attack by a methyl radical. Two explanations for the more effective progress of the reaction with CH_3B_{12} ester than with CH_3B_{12} are proposed. While CH_3B_{12} is present as a base-on compound under neutral conditions, CH_3B_{12}ester is present always in the base-off form. If we compare the Co(III)/Co (I) redox pair, the oxidation–reduction potential is more positive for the base-off compound than for the base-on compound. Thus, compared with methylcobalamin, CH_3B_{12}ester is more likely to undergo reduction. As a result, the progress of reductive demethylation becomes easier with CH_3B_{12}ester than with methylcobalamin. From the point of view of the chemistry of the coordination complexes, the cobalt atom of CH_3B_{12}ester lies elevated in the β plane above the plane of the corrin ring. Thus, it is more liable to undergo attack by reaction intermediates than methylcobalamine. As a result, methyl radical transfer to arsenic trioxide is easier from CH_3B_{12}ester than from CH_3B_{12}.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2008.12.002.

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