Supplementary Information

Experimental detail:

1. Reagents:

Ultra-pure water (18 M Ω /cm) produced by an ultra-pure water production device (Elix 3 UV, MIIIi-Q GradientA10, Nihon Millipore Ltd.) was used for the preparation of reagents, reference samples, and buffer solutions, and as solvent for the reactions. Reagents for arsenic reference solution (iAs^{III}, iAs^V, MMA, DMA, TMAO, TeMA, AB) were purchased from Tri Chemical Laboratories, Inc. (Yamanashi, Japan) and diluted with ultra-pure water. Naturally occurring vitamin B₁₂ (methylcobalamin) was obtained from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Biomimetic vitamin B₁₂ was synthesized according to the method previously reported.¹ Reduced glutathione (GSH, γ -Glu-Cys-Gly) was purchased from Sigma-Aldrich, Inc. Iodoacetic acid was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan).

To effect methylation, naturally occurring vitamin B_{12} , reductant, arsenic trioxide reference solution, and water were added to a Teflon-coated container and the mixture was heated in an oven. In the case of the biomimetic coenzyme B_{12} model, biomimetic B_{12} was dissolved in methanol before addition to the reaction mixture.

2. Methylation of arsenic trioxide by vitamin B₁₂ derivatives:

(1) Naturally occurring vitamin B₁₂

A screw-capped polypropylene (PP) tube was charged with GSH (20 mg, 65 μ mol) and methylcobalamin (5 mg, 3.7 μ mol), and Tris-hydrochloric acid buffer (pH 8; 50 μ L) was added. Then, 2 μ L of 100-ppm reference arsenic solution [iAs (III)] for atomic absorption spectroscopy was added (2.7 nmol of As), and the mixture was incubated in an oven at 100 °C for 2 h. The tube was cooled to room temperature and washed three times with 50 μ L of 1% nitric acid and three times with 200 μ L of ultra-pure water. The combined reaction mixture and washing solutions were placed in a 1.5 mL Eppendorf tube and diluted to 1 mL with ultra-pure water. The diluted solution (100 μ L) was mixed with 100 μ L of 10% hydrogen peroxide solution and heated at 50 °C for 1 h. The solution was finally cooled to room temperature, diluted to 1 mL with ultra-pure water, and analyzed by HPLC-ICP-MS.

(2) Biomimetic B₁₂

First, 4 M NaOH solution (20 μ L) was added to 10 μ L of methanol solution containing 5 mg of hydrophobic vitamin B₁₂. The mixture was stirred in a screw-capped PP tube and incubated in a

thermostated bath at 30 °C for 20 h. The solution was then neutralized with 6 M HCl solution and diluted with 10 μ L of ultra-pure water. GSH (20 mg, 65 μ mol) was added and the solution was stirred. From this step, the mixture was treated in the same way as the naturally occurring vitamin B₁₂.

3. Analysis:

(1) Anion-exchange column chromatography

An analytical sample comprising 20 μ L of the reaction mixture was treated with 10% hydrogen peroxide solution. The sample was applied to a column of anion-exchange resin (Hamilton PRP X100; 150 mm × 4.1 mm i.d., 3 μ m) with 10 mM NH₄H₂PO₄/10 mM NH₄NO₃, pH 6.3 as an eluent at a rate of 0.4 mL/min for separation by HPLC (Agilent 1100 series) (40°C). A qualitative and quantitative analysis of chemical species was performed by a direct online input of the measurements to an ICP-MS (Agilent 7500ce) and the signals from arsenic (*m*/*z* 75) were measured each second. The MS chromatogram obtained was quantified by using the software (ChemiStation) supplied as standard equipment with the ICP-MS.

(2) Cation-exchange column chromatography

The separation and quantification was conducted by the same HPLC-ICP-MS method as above, except that nitric acid buffer (5 mM nitric acid/6mM ammonium nitrate/1.5mM pyridinedicarboxylic acid) was used as the eluent and a cation-exchange resin (Shodex RSpak NN-414 (150 mm × 4.6 mm i.d., Tokyo, Japan) was used as the column.

Reference

1 Y. Murakami and Y. Hisaeda, Bull. Chem. Soc. Jpn., 1985, 58, 2652.