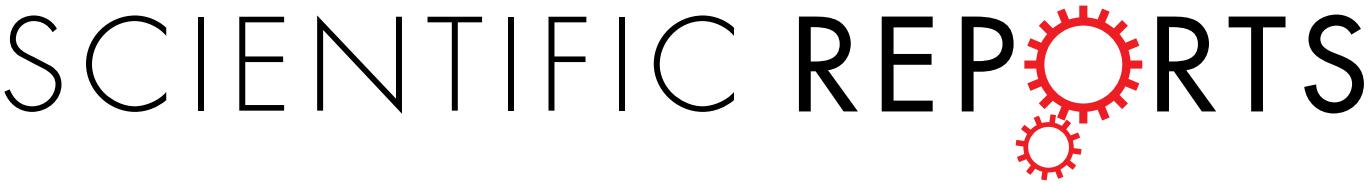

OPEN

Dimethylmercury Formation Mediated by Inorganic and Organic Reduced Sulfur Surfaces

Received: 08 March 2016
Accepted: 27 May 2016
Published: 15 June 2016

Sofi Jonsson[1,2], Nashaat M. Mazrui[1] & Robert P. Mason[1]

Underlying formation pathways of dimethylmercury ($(CH_3)_2Hg$) in the ocean are unknown. Early work proposed reactions of inorganic Hg (Hg^{II}) with methyl cobalamin or of dissolved monomethylmercury (CH_3Hg) with hydrogen sulfide as possible bacterial mediated or abiotic pathways. A significant fraction (up to 90%) of CH_3Hg in natural waters is however adsorbed to reduced sulfur groups on mineral or organic surfaces. We show that binding of CH_3Hg to such reactive sites facilitates the formation of $(CH_3)_2Hg$ by degradation of the adsorbed CH_3Hg. We demonstrate that the reaction can be mediated by different sulfide minerals, as well as by dithiols suggesting that e.g. reduced sulfur groups on mineral particles or on protein surfaces could mediate the reaction. The observed fraction of CH_3Hg methylated on sulfide mineral surfaces exceeded previously observed methylation rates of CH_3Hg to $(CH_3)_2Hg$ in seawaters and we suggest the pathway demonstrated here could account for much of the $(CH_3)_2Hg$ found in the ocean.

Dimethylmercury is a volatile and highly toxic form of mercury (Hg)[1]. It appears to be ubiquitous in marine waters and has been found in deep hypoxic oceanic water, coastal sediments and upwelling waters and in the mixed layer of the Arctic ocean[2–6]. Reported concentrations of $(CH_3)_2Hg$ in marine waters range from 0.01–0.4 pM and $(CH_3)_2Hg$ has been found to constitute a significant fraction (up to 80%) of the methylated Hg pool ($CH_3Hg + (CH_3)_2Hg$)[1,6]. The role of $(CH_3)_2Hg$ in the biogeochemical cycle of mercury, and its bioaccumulative potential, is not well known[6–8]. However, for oceanic systems, and for the marine boundary layer, it has been suggested that degradation of $(CH_3)_2Hg$ is an important source of CH_3Hg[5,9,10].

Monomethylmercury ($CH_3Hg^{II}X^{-1}$ where X is Cl^{-1}, OH^{-1}, $R\text{-}S^{-1}$ etc., here referred to as CH_3Hg) is known to bioaccumulate in aquatic food webs to concentrations of concern for human and wildlife health[1]. Understanding the methylation processes of Hg has thus been a key objective for comprehending the factors influencing its biogeochemical cycle. Formation of CH_3Hg and $(CH_3)_2Hg$ by aquatic organisms was first observed by Jensen and Jernelov in 1969[11]. A large number of bacterial strains have since been tested for their ability to methylate Hg, primarily focusing on CH_3Hg formation. A corrinoid type protein and a 2[4Fe-4S] ferredoxin protein encoded by the HgcA and HgcB gene, respectively, was recently identified as essential for CH_3Hg production by anaerobic bacteria[12]. The number of bacterial strains tested for their ability to methylate Hg to $(CH_3)_2Hg$ is however limited and the main process remains to be identified[13,14].

In culture studies with *Desulfovibrio desulfuricans,* Baldi and his coworkers, observed production of $(CH_3)_2Hg$ in paralell with a white precipitate following high additions of $CH_3Hg(aq)$[14]. This white precipitate was identified as bismethylmercury sulfide, $(CH_3Hg)_2S(s)$. Previous work had shown $(CH_3Hg)_2S(s)$ formation from the reaction between $CH_3Hg(aq)$ and H_2S, and with time, its degradation to metacinnabar (β-$HgS(s)$) and $(CH_3)_2Hg$[15]. Baldi and his coworkers thus suggested the production of $(CH_3)_2Hg$ by bacteria as an effect of sulfidogenic growth. Currently, the known pathways of $(CH_3)_2Hg$ formation relevant to field conditions include reaction of $CH_3Hg(aq)$ with H_2S[15] or selenoaminoacids[16] and methylation with methylcobalamin[17]. Computational calculations suggest a possible formation pathway from CH_3Hg complexed to L-cysteine, however experimental data is lacking[18]. With a up to 90% of the CH_3Hg in marine waters naturally occurring adsorbed to reduced sulfur groups on minerals

[1]Department of Marine Sciences, University of Connecticut, 1080 Shennecossett Road, Groton, CT06340, USA. [2]Centre for Environment and Sustainability, University of Gothenburg, Box 170, SE-405 30, Gothenburg, Sweden. Correspondence and requests for materials should be addressed to S.J. (email: sofijon84@gmail.com)

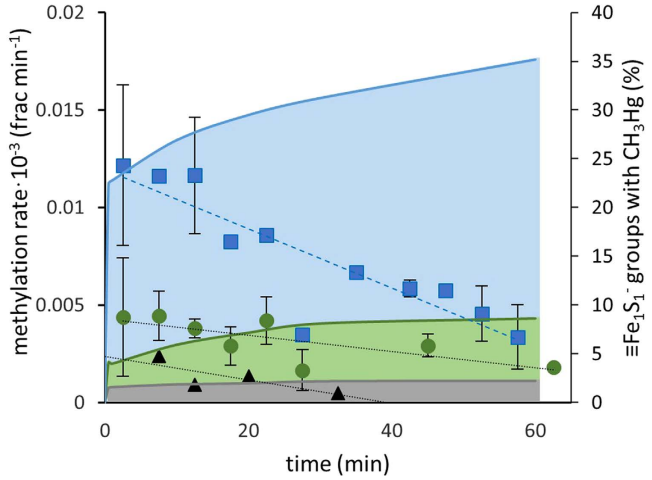

Figure 1. Methylation of CH_3Hg at different CH_3Hg:FeS_m*(s)* ratios. Methylation rate of CH_3Hg (fraction min^{-1}, scatter plot, left hand axis) and percent of $\equiv Fe_1S_1^-$ groups on the FeS_m*(s)* surface with CH_3Hg adsorbed (background area graph, right hand axis) at CH_3Hg:FeS_m*(s)* ratios (nmol μmol^{-1}) of 3.9 (blue squares, upper blue area), 1.0 (green circles, middle green area) and 0.25 (black triangles, lower gray area). Methylation rates at the three CH_3Hg:FeS_m*(s)* ratios tested were significantly different ($p < 0.05$, Analysis of Covariance).

or bound to thiols on organic matter, surface mediated processes are of interest. We therefore hypothesized that $(CH_3)_2Hg$ could be formed from CH_3Hg adsorbed to inorganic and organic reduced sulfur surfaces.

Result and Discussion

To test if $(CH_3)_2Hg$ could be formed from CH_3Hg on reduced sulfur surfaces, we initially adsorbed CH_3Hg to disordered Mackinawite (FeS_m*(s)*) in degassed purified water under low oxygen atmosphere and quantified the amount of $(CH_3)_2Hg$ formed. During the 1 h long experiment, we detected 0.37 ± 0.08 (0–20 min), 0.21 ± 0.07 (20–40 min) and 0.16 ± 0.07 (40–60 min) pmol of $(CH_3)_2Hg$ formed from 2.3 nmol of CH_3Hg (Supplementary Table S1). Control experiments with water and filtered FeS_m*(s)* slurry (0.02 μm) did not produce detectable levels of $(CH_3)_2Hg$ supporting its formation from CH_3Hg adsorbed onto FeS_m*(s)* particles.

In the present experiment, CH_3Hg was the only methyl containing compound and therefore acted as both the methyl donor and acceptor. The reaction could therefore involve either two CH_3Hg molecules adsorbed on neighboring sulfide groups or one molecule adsorbed reacting with a molecule in solution. To test this, we measured the formation of $(CH_3)_2Hg$ at CH_3Hg:FeS_m*(s)* ratios (nmol·μmol^{-1}) of 6.1, 1.8 and 0.38 by varying the concentration of CH_3Hg. FeS_m*(s)* has been described as having a surface dominated by equal moles of mono and tri coordinated sulfide groups with the mono coordinated sulfide ($\equiv Fe_1S_1^-$) having stronger anionic properties[19]. We therefore assume these are the primary sites of CH_3Hg adsorption and calculated the fraction of $\equiv Fe_1S_1^-$ groups occupied by CH_3Hg in the experiment. We observed a greater fraction of CH_3Hg methylated at higher CH_3Hg:FeS_m*(s)* ratios; i.e. where a higher percent of $\equiv Fe_1S_1^-$ sites are saturated (Fig. 1). The fraction of CH_3Hg in solution did not differ between the tests (Supplementary Fig. S1). This suggests the fraction methylated to be dependent on the number of sites occupied rather than concentration of CH_3Hg remaining in solution. Additional experiments covering a wider range of CH_3Hg:FeS_m*(s)* ratios ($3.4 \cdot 10^{-2}$ to $3.4 \cdot 10^4$ nmol·μmol^{-1}), obtained by varying the concentration of FeS_m*(s)*), demonstrated that the fraction of CH_3Hg that was methylated increased as more $\equiv Fe_1S_1^-$ sites were occupied, and then decreased after the number of $\equiv Fe_1S_1^-$ groups were saturated with CH_3Hg, and the fraction of CH_3Hg bound decreased (Fig. 2). Both our experiments thus support a reaction mechanism involving two CH_3Hg molecules adsorbed on neighboring sulfide groups rather than a reaction involving one CH_3Hg molecule adsorbed on the surface and one molecule in solution.

FeS_m*(s)* is the first mineral formed from environmental precipitation of S^{2-}*(aq)* and Fe^{2+} *(aq);* e.g. in sediment pore water and inside bacterial cells, and is the precursor to more stable FeS forms; e.g, greigite (Fe_3S_4*(s)*) and pyrite (FeS_2*(s)*)[20]. Experiments with other, more stable, sulfide minerals (CdS*(s)* and HgS*(s)*), showed similar fractions of CH_3Hg conversion to $(CH_3)_2Hg$ suggesting that the internal stability of the mineral is of minor importance (Supplementary Table S2). In a similar manner, the aging of FeS_m*(s)* did not affect the fraction methylated (Supplementary Table S3).

Based on the above discussed results, we propose a S_N2-type reaction for the formation of $(CH_3)_2Hg$ from CH_3Hg adsorbed onto sulfide mineral surfaces (Fig. 3). After adsorption of CH_3Hg onto the surface, the reaction is initiated by a nucleophilic attack of one of the CH_3Hg holding sulfur atoms on a Hg atom of a CH_3Hg molecule adsorbed on a neighboring sulfide site. The intermediate formed is then rearranged resulting in, as final products, one $(CH_3)_2Hg$ molecule and incorporation (co-precipitation) of the other Hg atom, becoming bound to two sulfur atoms at the surface of the sulfide mineral. For the reaction of CH_3Hg with FeS_m*(s),* previous spectroscopic studies of Hg^{2+} adsorbed to FeS_m*(s)* suggest that the Hg atom could either remain on the surface of the mineral or be precipitated as metacinnabar, β-HgS*(s)* (and Fe^{2+} be released into the solution)[21,22]. Which of these two end products would dominate in our experiment remains unclear as the final presumed Hg^{2+} :FeS_m*(s)* is

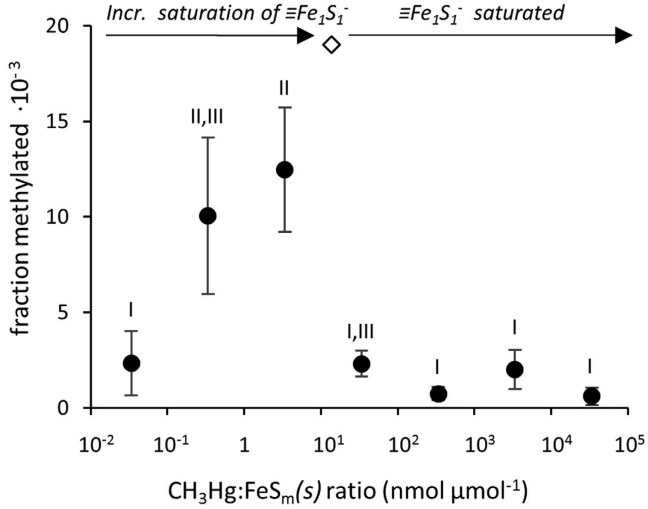

Figure 2. Methylation of CH_3Hg at different CH_3Hg:FeS_m*(s)* ratios. Fraction of CH_3Hg methylated at CH_3Hg:FeS_m*(s)* ratios (nmol µmol^{-1}) of $3.4{\cdot}10^{-2}$ to $3.4{\cdot}10^{4}$ and the theoretical saturation point of $\equiv Fe_1S_1^-$ groups on the FeS_m*(s)* surface (diamond). Roman numbers indicate significant differences ($p < 0.05$).

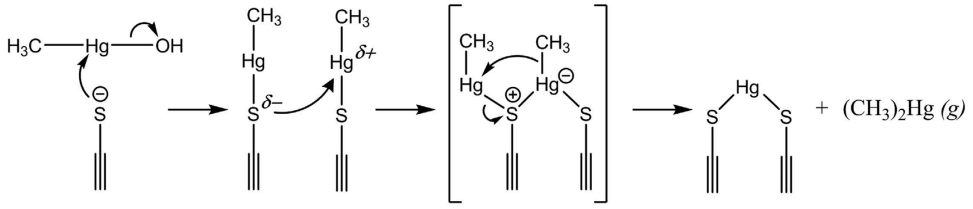

Figure 3. Proposed reaction mechanism. The proposed S_N2-type reaction mechanism for the formation of $(CH_3)_2Hg$ from CH_3Hg mediated by inorganic or organic surfaces with neighboring reduced sulfur groups.

lower than in the previous studies, and furthermore, a significant fraction of added Hg is likely still remaining as CH_3Hg adsorbed onto the FeS_m*(s)*. Calculations of the equilibrium constant and the ΔG for the overall reaction of CH_3Hg and FeS_m*(s)* with $(CH_3)_2Hg$ and HgS*(s)* as end-products supports that the reaction is thermodynamically favorable (see supplementary discussion).

We also tested the previously demonstrated methylation pathway involving CH_3Hg*(aq)* and dissolved sulfide[15] and compared it to the reaction mediated by FeS_m*(s)*. The ratio CH_3Hg:S^{2-} was varied from the optimum molar ratio of 2 ($2\ CH_3Hg(aq) + S^{2-}(aq) \rightarrow HgS(s) + (CH_3)_2Hg(g)$) to that matching the CH_3Hg:FeS_m*(s)* experiments (4.3 nmol µmol^{-1}). The fraction CH_3Hg methylated with S^{2-}*(aq)* was 6–40 times lower than the fraction methylated on FeS_m*(s)* (Fig. 4). The geometry of the $(CH_3Hg)_2S$ molecule should theoretically limit the transfer of the methyl group between Hg atoms bound to the same S (given the linearity of the S-Hg-C bond). We found the activation energy, E_a, for the formation of $(CH_3)_2Hg$ from the reaction of CH_3Hg with S^{2-}*(aq)* and FeS_m*(s)* to be 41 ± 6.8 and 91 ± 4.6 kJ mol^{-1}, respectively (Supplementary Fig. S2). This suggests that the reaction with dissolved sulfide is slower due to a limited number of CH_3Hg molecules close enough for transfer of the methyl group to occur. The previously proposed reaction mechanism for the observed formation of $(CH_3)_2Hg$ in pure cultures of sulfate reducing bacteria, and in sediment amended with CH_3Hg*(aq)* and purged with H_2S*(g)*, involves $(CH_3Hg)_2S$*(s)* as an intermediate[14,15]. Given that surfaces with reduced sulfide would also be present in such experimental systems, we suggest that even though $(CH_3Hg)_2S$*(s)* has been observed when reacting CH_3Hg*(aq)* with H_2S in water and when CH_3Hg was added to a subsample of the cell cultures, formation of $(CH_3)_2Hg$ by the mechanism proposed here is also a possible explanation for the $(CH_3)_2Hg$ produced in those previous experiments.

Experiments conducted from pH 6 at which most $\equiv Fe_1S_1^-$ groups would be protonated, to pH 8 where they would be deprotonated[19], showed no difference in $(CH_3)_2Hg$ formation (Supplementary Fig. S3). Further, experiments conducted at ionic strengths of 0.017 and 0.20 M (NaCl) demonstrated that ionic strength did not impact the methylation. Adsorption studies of inorganic Hg onto FeS_m*(s)* at different pH levels have shown no significant difference in the amount of inorganic Hg adsorbed even though small differences in the dissolved fraction were observed[23]. Our results showing that $(CH_3)_2Hg$ formation rate is independent of pH and ionic strength are consistent with the high binding capacity of FeS_m*(s)* for Hg compounds in both acidic and basic conditions and the fact that the reaction between CH_3Hg and FeS_m*(s)* is a surface mediated process.

Reactive sites containing multiple thiol groups located on the surface of proteins are known to be important adsorption sites for heavy metals, including Hg[24]. To test if $(CH_3)_2Hg$ could also be formed from CH_3Hg

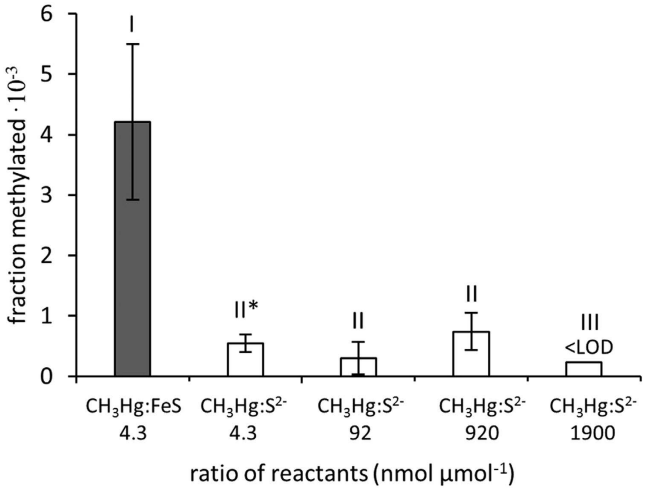

Figure 4. Methylation of CH_3Hg with S^{2-}*(aq)*. Fraction of CH_3Hg methylated on FeS_m*(s)* (±SD, n = 3) at a CH_3Hg:FeS_m*(s)* ratio of 4.3 (nmol µmol^{-1}) or with dissolved sulfide at CH_3Hg:S^{2-} ratios of 4.3 to 1900 (nmol µmol^{-1}). LOD = Limit of detection. Roman numbers indicate significant differences ($p < 0.05$). *One outlier removed (n = 2).

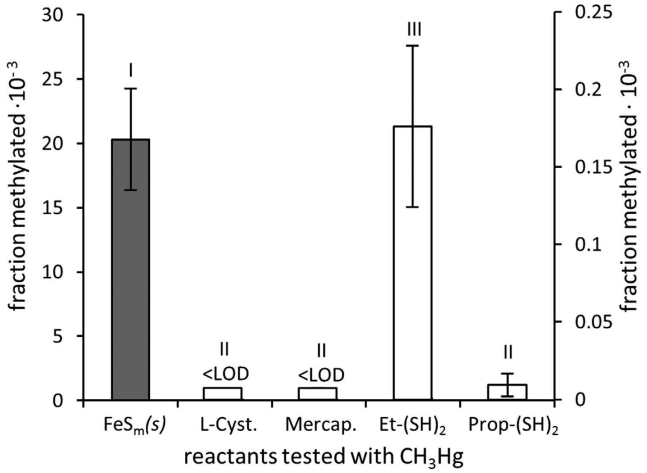

Figure 5. Methylation of CH_3Hg complexed with organic thiols. Fraction of CH_3Hg methylated (±SD, n = 3) on FeS_m*(s)* (CH_3Hg:FeS_m*(s)* ratio of 3.4 nmol µmol^{-1}, left hand axis) or with L-Cysteine (L-Cyst.), 3-mercaptopropionic acid (Mercap.), 1,2-ethanedithiol (Et-$(SH)_2$) or 1,3-propanedithiol (Prop-$(SH)_2$) (CH_3Hg:thiol ratio of 1000 and 2000 nmol µmol^{-1} for mono- and dithiols respectively giving a CH_3Hg:R-SH ratio of 1 for both mono and di-thiols, right hand axis), right hand axis). LOD = Limit of detection. Roman numbers indicate significant differences ($p < 0.05$).

adsorbed onto neighboring thiol groups, we reacted CH_3Hg with two dithiol compounds (1,2-ethanedithiol and 1,3-propanedithiol) and two monothiol compounds (L-cysteine and 3-mercaptopropionic acid) at CH_3Hg:R-SH ratios of 1:1. We detected methylation of CH_3Hg using the dithiols but not with the monothiols (i.e. fraction methylated $<7.2 \cdot 10^{-6}$) (Fig. 5). The higher methylation observed using 1,2-ethanedithiol compared to 1,3-propanedithiol could be due to a longer distance between the thiols of the latter. The fraction of CH_3Hg being methylated was two orders of magnitude lower when the reaction was mediated by 1,2-ethanedithiol compared to FeS_m*(s)*. The sulfidic mineral surfaces will have a higher density of electrons in comparison to alkane dithiols, which should be favorable for the proposed reaction (Fig. 3). We used simple organic dithiols here as analogs for protein sites with multiple thiol groups as previous work have shown Hg^{2+} to complex proteins and natural organic matter via thiol groups as a bicoordinated complex (RS-Hg-SR)[24,25]. The reactivity and symmetry (which is likely more flexible in proteins) are likely different between alkane dithiols and active sites on proteins. Nonetheless, our results show the potential for the formation of $(CH_3)_2Hg$ from CH_3Hg adsorbed on neighboring protein thiol groups. We speculate that the higher methylation rate mediated by sulfide minerals suggests that this reaction could be more favorable on iron sulfur clusters (e.g. Fe_2S_2, Fe_3S_4, Fe_4S_4) present in certain proteins[26,27] compared to protein thiols. We examined the potential for the reaction to occur in artificial sea water

in presence of diatom algae cells (*Thalassiosira weissflogii*) by comparing the formation of $(CH_3)_2Hg$ in pure sea water or in sea water with the presence of whole cells, cellular membrane material and organelles (i.e. nuclei and mitochondria settled at the g forces used here), or the remaining cytoplasm (Supplementary Fig. S4). In all cases, while $(CH_3)_2Hg$ was formed, the rate of formation was lower (8.6, 1.4 and 2.5 times in the presence of whole cells, cellular membrane material and organelles, respectively) than for FeS_m*(s)* in seawater without organic matter present. The lower methylation in presence of plankton organic matter may be the result of an increase in the competition of CH_3Hg binding to sites less reactive for the methylation process. The results however demonstrate the potential for the above outlined mechanism to occur on FeS-clusters within cells after assimilation of CH_3Hg from marine waters.

Our study is the first to demonstrate the formation of $(CH_3)_2Hg$ from CH_3Hg adsorbed onto sulfide mineral surfaces or organic dithiols (CH_3Hg methylation rates up to $0.012 \pm 0.004 \times 10^{-3}$ detected, Fig. 1). In the ocean, the highest concentrations of $(CH_3)_2Hg$ are typically found in low oxygen environments where active degradation of organic matter is occurring, or in regions of concentrated biological material, as well as in the deep ocean[1,2]. The relatively high degradation rate of $(CH_3)_2Hg$ observed in marine waters suggests it must be continually produced in the water column, sediments or in association with hydrothermal systems[7]. The formation of $(CH_3)_2Hg$ has mainly been hypothesized to be bacterially mediated, however direct experimental support for this assertion is missing[28]. Further, the *in vivo* mechanism by which the $(CH_3)_2Hg$ could be produced inside the bacterial cells has not been identified[14]. We propose that the reaction pathway discussed above may be important abiotic as well as biotic pathways for formation of $(CH_3)_2Hg$ in the oceanic system. In addition to methylation of CH_3Hg in the presence of biological material via pathway involving the binding of CH_3Hg to thiols and the proposed methyl transfer reactions outlined above, there is also the potential for these reactions to occur in the presence of metal-sulfide particles within marine aggregates, or in the sulfide particles that are associated with hydrothermal vent plumes. The presence of reduced sulfur in the upper ocean has been shown in numerous studies[29,30]. There is also evidence for reducing conditions within sinking marine aggregates, and the presence of CdS*(s)* in low oxygen sub-thermocline ocean waters[31,32]. Finally, there is substantial evidence for metal sulfide and pyrite particulates emanating from hydrothermal vents[33]. Although the concentrations of CH_3Hg in our experiments exceed the concentrations found in marine waters, our ratio of CH_3Hg to sulfide mineral surface area are similar to the ratio expected on particles or inside planktonic cells present in the ocean. For example, for the low oxygen waters in the North Atlantic, where observed concentration of particulate Cd has been assumed to mainly be composed of CdS*(s)*, calculated particulate CH_3Hg:Cd is about 10^{-3} (molar basis)[34,35]. Furthermore, inside planktonic cells the molar CH_3Hg:Fe ratio of 10^{-3} to 10^{-4} is typically found but the expected CH_3Hg:FeS is lower given that not all intracelleular Fe would occur as FeS-clusters[8,36,37]. Reported rates of $(CH_3)_2Hg$ formation in marine water are scant. Lehnherr *et al.* reported potential CH_3Hg methylation rates, producing $(CH_3)_2Hg$, of up to $1.6{\cdot}10^{-3}$ d^{-1} for Arctic waters[28]. The fraction of CH_3Hg converted to $(CH_3)_2Hg$ in our experiments (up to $20{\cdot}10^{-3}$ in purified water and up to $15{\cdot}10^{-3}$ in artificial sea water, Fig. 5 and Supplementary Fig. S4) for experiments carried out within 24 h are an order of magnitude higher than the methylation rates observed by Lehnherr *et al.* We propose that the reactions outlined above could produce a significant portion of the $(CH_3)_2Hg$ within the upper ocean water column, primarily in association with organic matter recycling. In the deep ocean, the elevated concentrations of total Hg, CH_3Hg, as well as dissolved and colloidal Fe, found during the Geotraces GA03 cruise in the vicinity of the mid-Atlantic Ridge[34,38], compared to other deep ocean waters, suggest that hydrothermal vent plumes are environments where $(CH_3)_2Hg$ could be formed by reactions mediated by FeS surfaces.

Material and Methods

The preparation of sulfide minerals and all experiments were conducted under a N_2*(g)* or Ar*(g)* atmosphere and using degassed (N_2*(g)* or Ar*(g)* purged) purified water ($\Omega < 18.2$). Disordered Mackinawite (FeS_m*(s)*) was prepared by adding 100 ml of 0.6 M Na_2S to 100 ml of 0.6 M Morh's salt ($(NH_4)_2Fe(II)(SO_4)_2{\cdot}6H_2O$)[39]. The precipitated crystals of FeS_m*(s)* were aged for 0 h, 1 day and 7 days, then collected by centrifugation (5 min, 2.6 kG) and washed three times with purified water. Since the aging of FeS_m*(s)* has previously been shown to significantly stop at −80 °C[39], the final product was re-suspended in water, subsampled into smaller vials and stored in a N_2 atmosphere at −80 °C until use. For experiments where the activity of FeS_m*(s)* was compared to that of CdS*(s)* and HgS*(s)* (Supplementary Table S2), FeS_m*(s)* was prepared as described above (25 ml of 0.6 M Na_2S and 0.6 M of Morh's salt), and at the same time, CdS*(s)* and HgS*(s)* were synthesized by adding 25 ml or 15 ml of 0.6 M Na_2S to 25 ml of 0.6 M $Cd(NO_3)_2{\cdot}4\,H_2O$ or 15 ml 0.6 M $HgCl_2$ (prepared by dissolving $HgCl_2$*(s)* using 700 μL HCl following dilution in purified water), respectively. For CdS*(s)* precipitated with excess of Cd, this was prepared by adding 12.5 ml of 0.6 M Na_2S and 12.5 ml degassed MQ water to 25 ml 0.6 M $Cd(NO_3)_2{\cdot}4\,H_2O$. The precipitated crystals were collected by centrifugation (5 min, 2.6 kG), and washed four times until excess acid in the HgS*(s)* slurry was removed (pH ~7). A subsample of each was freeze-dried to calculate the concentration (weight to weight) of stock slurries, and characterized using X-ray Diffraction Cristallography (XRD) and Brunauer–Emmett–Teller (BET) (Supplementary Table S4, Fig. S5 and discussion).

Formation of $(CH_3)_2Hg$ was tested by adding CH_3Hg*(aq)* to FeS_m*(s)*, CdS*(s)*, HgS*(s)*, S^{2-}*(aq)*, L-Cysteine, 3-mercaptopropionic acid, 1,2-ethanedithiol or 1,3-propanedithiol in acid cleaned glass vials (total volume of 42 cm^3). The amount of thiol ligand, CH_3Hg and final volume of solution used is summarized in Supplementary Table S5. Each experimental set was done in triplicate and the CH_3Hg(*aq*) standard was prepared from a 1000 ppm CH_3Hg(*aq*) stock solution (pH 1, Alfa Aesar) and pH was adjusted to ~6–8 using 2–8 M KOH(*aq*). The produced $(CH_3)_2Hg$(*g*) was collected onto Carbotrap™ (Supelco) solid absorbent either by purging the headspace of the vial with 200 ml/min of Argon (Ar) while gently stirring the solution with a magnetic stirring bar (results presented as formation rates, i.e. $n(CH_3)_2Hg(g)$ (pmol) · nCH_3Hg added $(pmol)^{-1}$ · time $(min)^{-1}$), or by sampling 0.1–5 ml of the headspace from a closed vial through the septa using a syringe. For the latter, the total concentration of $(CH_3)_2Hg$ was calculated based on the relative volumes of water and gas, the sampled volume of gas and the dimensionless

Henry solubility constant (H^{cc}; concentration in the aqueous phase · concentration in the gas phase^{-1}) for $(CH_3)_2Hg$[40], and results are presented as fraction of CH_3Hg methylated (i.e. n$(CH_3)_2Hg$(g) (pmol) · nCH_3Hg added (pmol)$^{-1}$). In the initial experiment, the $FeS_m(s)$ slurry was filtered through a 0.02 um PTFE syringe filter and the control experiment done by adding 2.3 nmol of CH_3Hg to 1 ml of the filtrate. The percent of $\equiv Fe_1S_1^-$ with CH_3Hg adsorbed was calculated from the concentration of $CH_3Hg(aq)$ immobilized in a separate adsorption experiment (Supplementary Fig. S1), the specific surface area of $FeS_m(s)$ (Supplementary Table 3) and assuming two $\equiv Fe_1S_1^-$-groups nm^{-2} [19]. The activation energy, E_a (kJ/mol), for the formation of $(CH_3)_2Hg$ was determined assuming a pseudo first order reaction and using the Arrhenius Equation ($\ln k = \ln Ae - E_a/RT$; rate constant (k), frequency factor (Ae), activation energy (E_a), gas constant (R), temperature (T; in kelvin) from experiments conducted at 0, 18, 40 and 60 °C (n = 3, details are provided in Supplementary Table S5). The activation energy (including standard deviation) was calculated from the slope of ln k vs. 1/T (slope = $-E_a/R$). For CH_3Hg on $FeS_m(s)$, no $(CH_3)_2Hg(g)$ was detected in samples incubated at 0 °C hence the production of $(CH_3)_2Hg$ during the cooling process was neglected. For the reaction with S^{2-} (where a higher concentration of CH_3Hg was used), the $(CH_3)_2Hg$ formed was similar at 0 and 18 °C. The E_a was thus calculated only using the results obtained at 40 and 60 °C.

When the reaction vessels were purged, sampled gas was first dried on a soda lime trap placed in line with the Carbotrap™ column, and when the headspace was sampled, the gas was injected directly on the Carbotrap™ column via an injection valve. Collected $(CH_3)_2Hg(g)$ was then thermally desorbed and separated by isothermal gas chromatography before being pyrolytically decomposed to Hg^0 and detected using CVAFS (Tekran, model 2500). External calibration was done using known amounts of synthesized $(CH_3)_2{}^{200}Hg(aq)$ standard purged onto Carbotrap™ columns. The $(CH_3)_2{}^{200}Hg$ was manufactured in house from $^{200}HgCl_2$ and 3 M methyl magnesium chloride in tetrahydrofuran (Supplementary Method). WARNING, Extreme caution is needed when synthesizing $(CH_3)_2Hg$ as it is a volatile and extremely toxic compound! Even small amounts absorbed through the skin have proven fatal! Due to variations in the concentration of $(CH_3)_2Hg$ in the diluted aqueous standard prepared from synthesized stock solution, standards were prepared daily and the concentration was determined by collecting purgeable Hg from the standard on to a gold trap and using a second calibration of 10–200 ul of $Hg^0(g)$ at a known temperature (also purged onto gold traps). Detection limits were calculated from the amount of $(CH_3)_2Hg$ detected from experimental replicates utilizing equimolar concentrations of $CH_3Hg(aq)$ (n = 3, mean + 2 SD).

Adsorption of $CH_3Hg(aq)$ onto $FeS_m(s)$ was tested by incubating 0.70 (n = 1), 2.8 (n = 1) and 11 (n = 1) nmol of CH_3Hg with 2.8 umol $FeS_m(s)$ in 0.6 ml of DI in a disposable syringe. The samples were left for up to 60 minutes and the dissolved fraction was then collected using a 0.02 um syringe filter. In a second experiment, 0.34 nmol CH_3Hg was added to 50 μmol of $FeS_m(s)$ in 10 ml degassed DI. The samples were filtered after an equilibration time of 10 min, 60 min or 24 h using 0.05 um membrane filters. The amount of CH_3Hg remaining in solution was quantified using EPA method 1630 with an automated analyzer (Tekran 2700). Statistical analysis was conducted using IBM SPSS statistics. All data (fraction methylated or methylation rates) were log transformed before analysis of variance followed by Tukey's post-hoc test. For non-normally distributed log transformed data, median test following a pairwise t-test approach was performed.

References

1. Fitzgerald, W. F., Lamborg, C. H. & Hammerschmidt, C. R. Marine Biogeochemical Cycling of Mercury. *Chem. Rev.* **107,** 641–662 (2007).
2. Mason, R. P. & Fitzgerald, W. F. Alkylmercury species in the equatorial Pacific. *Nature* **347,** 457–459 (1990).
3. Cossa, D., Coquery, M., Gobeil, C. & Martin, J.-M. In *Reg. Glob. cycles Mercur. Sources, fluxes, mass Balanc*. (Baeyens, R., Edinghaus, R. & Vasiliev, O.) 229–247 (1996).
4. Conaway, C. H. *et al.* Dimethylmercury in coastal upwelling waters, Monterey Bay, California. *Environ. Sci. Technol.* **43,** 1305–1309 (2009).
5. Mason, R. P. *et al.* Mercury biogeochemical cycling in the ocean and policy implications. *Environ. Res.* **119,** 101–117 (2012).
6. Kirk, J. L. *et al.* Mercury in Arctic marine ecosystems: sources, pathways and exposure. *Environ. Res.* **119,** 64–87 (2012).
7. Morel, F. M. M., Kraepiel, A. M. L. & Amyot, M. The Chemical Cycle and Bioaccumulation of Mercury. *Annu. Rev. Ecol. Syst.* **29,** 543–566 (1998).
8. Mason, R. P., Reinfelder, J. R. & Morel, F. M. M. Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. *Environ. Sci. Technol.* **30,** 1835–1845 (1996).
9. Mason, R. P. & Fitzgerald, W. F. The distribution and biogeochemical cycling of mercury in the equatorial Pacific Ocean. *Deep Sea Res. Part I* **40,** 1897–1924 (1993).
10. Baya, P. A., Gosselin, M., Lehnherr, I., Louis, V. L. S. & Hintelmann, H. Determination of monomethylmercury and dimethylmercury in the arctic marine boundary layer. *Environ. Sci. Technol.* **49,** 223–232 (2015).
11. Jensen, S. & Jernelov, A. Biological methylation of mercury in aquatic organisms. *Nature* **223,** 753–754 (1969).
12. Parks, J. M. *et al.* The genetic basis for bacterial mercury methylation. *Science* **339,** 1332–1335 (2013).
13. Benoit, J. M., Gilmour, C. C. & Mason, R. P. Aspects of bioavailability of mercury for methylation in pure cultures of desulfobulbus propionicus (1pr3). *Appl. Environ. Microbiol.* **67,** 51–58 (2001).
14. Baldi, F., Pepi, M. & Filippelli, M. Methylmercury Resistance in Desulfovibrio desulfuricans Strains in Relation to Methylmercury Degradation. *Appl. Environ. Microbiol.* **59,** 2479–2485 (1993).
15. Craig, P. J. & Bartlett, P. D. The role of hydrogen sulfide in environmental transport of mercury. *Nature* **275,** 635–637 (1978).
16. Khan, M. A. & Wang, F. Chemical demethylation of methylmercury by selenoamino acids. *Chem. Res. Toxicol.* **23,** 1202–1206 (2010).
17. Imura, N., Sukegawa, E., Pan, S.-K., Nagao, K. & Kim, J.-Y. Methylation of inorganic mercury with methylcobalamin, a vitamin B12 analog. *Science.* **172,** 1248–1249 (1971).
18. Asaduzzaman, A. M. & Schreckenbach, G. Degradation mechanism of methyl mercury selenoamino acid complexes: a computational study. *Inorg. Chem.* **50,** 2366–2372 (2011).
19. Wolthers, M., Charlet, L., van Der Linde, P. R., Rickard, D. & van Der Weijden, C. H. Surface chemistry of disordered mackinawite (FeS). *Geochim. Cosmochim. Acta* **69,** 3469–3481 (2005).
20. Rickard, D. & Luther, G. W. *Chemistry of iron sulfides*. *Chem. Rev.* **107** (2007).
21. Skyllberg, U. & Drott, A. Competition between disordered iron sulfide and natural organic matter associated thiols for mercury(II)-an EXAFS study. *Environ. Sci. Technol.* **44,** 1254–1259 (2010).

22. Jeong, H. Y., Klaue, B., Blum, J. D. & Hayes, K. F. Sorption of mercuric ion by synthetic nanocrystalline mackinawite (FeS). *Environ. Sci. Technol.* **41,** 7699–7705 (2007).
23. Liu, J., Valsaraj, K. T., Devai, I. & DeLaune, R. D. Immobilization of aqueous Hg(II) by mackinawite (FeS). *J. Hazard. Mater.* **157,** 432–440 (2008).
24. DeSilva, T. M., Veglia, G., Porcelli, F., Prantner, A. M. & Opella, S. J. Selectivity in heavy metal- binding to peptides and proteins. *Biopolymers* **64,** 189–197 (2002).
25. Skyllberg, U., Bloom, P. R., Qian, J., Lin, C. M. & Bleam, W. F. Complexation of mercury(II) in soil organic matter: EXAFS evidence for linear two-coordination with reduced sulfur groups. *Environ. Sci. Technol.* **40,** 4174–4180 (2006).
26. Beinert, H., Holm, R. H. & Munch, E. Iron-sulfur clusters : nature's modular, multipurpose structures. *Science.* **277,** 653–659 (1997).
27. Rouault, T. A. & Tong, W.-H. Iron-sulphur cluster biogenesis and mitochondrial iron homeostasis. *Nat. Rev. Mol. Cell Biol.* **6,** 345–351 (2005).
28. Lehnherr, I., St. Louis, V. L., Hintelmann, H. & Kirk, J. L. Methylation of inorganic mercury in polar marine waters. *Nat. Geosci.* **4,** 298–302 (2011).
29. Cutter, G. A. & Krahforst, C. F. Sulfide in surface waters of the western Atlantic ocean. *Geophys. Res. Lett.* **15,** 1393–1396 (1988).
30. Luther, G. W. & Tsamakis, E. Concentration and form of dissolved sulfide in the oxic water column of the ocean. *Mar. Chem.* **27,** 165–177 (1989).
31. Janssen, D. J. *et al.* Undocumented water column sink for cadmium in open ocean oxygen-deficient zones. *Proc. Natl. Acad. Sci.* **111,** 6888–6893 (2014).
32. Balzano, S., Statham, P. J., Pancost, R. D. & Lloyd, J. R. Role of microbial populations in the release of reduced iron to the water column from marine aggregates. *Aquat. Microb. Ecol.* **54,** 291–303 (2009).
33. Yücel, M., Gartman, A., Chan, C. S. & Luther, G. W. Hydrothermal vents as a kinetically stable source of iron-sulphide-bearing nanoparticles to the ocean. *Nat. Geosci.* **4,** 367–371 (2011).
34. Bowman, K. L., Hammerschmidt, C. R., Lamborg, C. H. & Swarr, G. Mercury in the North Atlantic Ocean: The U.S. GEOTRACES zonal and meridional sections. *Deep. Res. Part II Top. Stud. Oceanogr.* **116,** 251–261 (2015).
35. Conway, T. M. & John, S. G. The cycling of iron, zinc and cadmium in the North East Pacific Ocean – Insights from stable isotopes. *Geochim. Cosmochim. Acta* **164,** 262–283 (2015).
36. Luengen, A. C. & Flegal, A. R. Role of phytoplankton in mercury cycling in the San Francisco Bay estuary. *Limnol. Oceanogr.* **54,** 23–40 (2009).
37. Eisler, R. *Compendium of Trace Metals and Marine Biota*. (Elsevier, 2009).
38. Fitzsimmons, J. N. *et al.* Partitioning of dissolved iron and iron isotopes into soluble and colloidal phases along the GA03 GEOTRACES North Atlantic Transect. *Deep. Res. Part II* **116,** 130–151 (2015).
39. Wolthers, M., Van Der Gaast, S. J. & Rickard, D. The structure of disordered mackinawite. *Am. Mineral.* **88,** 2007–2015 (2003).
40. Talmi, Y. & Mesmer, R. E. Studies on vaporization and halogen decomposition of methylmercury compounds using gc with a microwave detector. *Water Res.* 547–552 (1975).

Acknowledgements

This research was supported by the Swedish Research Council (International Postdoc grant 637-2014-54) to S.J, and partial support for N.M.M and R.P.M from the National Institutes of Health, through collaboration with investigators at Dartmouth College (NIH Grant Number P42 ES007373). We thank John Macharia and Andrew Meguerdichian in Dr. Steven Suib's group at the University of Connecticut for BET and XRD analysis. Axenic plankton culture was provided by the National Oceanic and Atmospheric Administration, Milford, US.

Author Contributions

S.J. and N.M.M. designed and carried out the experimental work; R.P.M. supervised the research; S.J., N.M.M. and R.P.M. wrote the paper.

Additional Information

Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Jonsson, S. *et al.* Dimethylmercury Formation Mediated by Inorganic and Organic Reduced Sulfur Surfaces. *Sci. Rep.* **6**, 27958; doi: 10.1038/srep27958 (2016).