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Degradation Mechanism of Methyl Mercury Selenoamino Acid Complexes: A Computational Study

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Supporting Information

ABSTRACT: Density functional theory (DFT) calculations have been carried out on the possible degradation/demethylation mechanism of methyl mercury (CH_3Hg^+) complexes with free cysteine and seleonocysteine. The binding of CH_3Hg^+ ions with one (seleno)amino acid is thermodynamically favorable. However, the binding with another acid molecule is a highly unfavorable process. The CH_3Hg-(seleno)cysteinate then degrades to bis(methylmercuric)sulphide (selenide for the Se-containing complex) which in turn forms dimethyl mercury and HgS/HgSe, the latter being precipitated out as nanoparticles. The dimethyl mercury interacts with water molecules and regenerates the CH_3HgOH precursor. The calculated free energies of formation confirm the thermodynamic feasibility of every intermediate step of the degradation cycle and fully support earlier experimental results. In completing the cycle, one unit of mercury precipitates out from two units of sources, and thereby Se antagonizes the Hg toxicity. The degradation of CH_3Hg-L-cysteinate is thermodynamically more favorable than the formation of CH_3Hg-L-cysteinate. The preferred degradation of the CH_3Hg-L-cysteinate suggests that another mechanism for CH_3Hg to cross the blood−brain barrier should exist.

INTRODUCTION

The toxicological effects of mercury (Hg) in humans and other mammals have been known for decades. While inorganic Hg can cause injury to kidneys, livers, and lungs,[1] organic Hg, particularly monomethylmercury (CH_3Hg^+ and its complexes; referred to as CH_3Hg hereafter), can cross the blood−brain barrier and cause irreversible damage to the central nervous system tissue.[2−10] One of the most likely reasons for the Hg toxicity is the affinity of Hg to the sulfur atom of sulfuramino acids. For instance, CH_3Hg-L-cysteinate[2] is thought to be the main CH_3Hg species that is transported by the amino acid transport system across the blood−brain barrier where it subsequently exerts its neurotoxicity.

The fate of the organomercurials in biological systems, however, is not very clear. Due to the kinetic stability of the Hg−C bond,[11,12] the degradation or demethylation of CH_3Hg in the aquatic environment is thought to happen via chemical, photolysis, or microbial processes.[12,13] One chemical demethylation pathway in nature is the reaction between CH_3Hg and H_2S via a bis(methylmercuric)sulphide intermediate, HgS(s) being the end product.[14,15] Recently a new chemical demethylation pathway was discovered which involves the reaction between CH_3Hg and (seleno)amino acids with bis(methylmercuric)-selenide as an intermediate and HgSe(s) the end product.[12] The In Vivo presence of HgS(s) and HgSe(s) has been analytically confirmed in rat plasma, brain, tissues, liver, and gastrointestinal tract.[16−20]

The challenge in validating both chemical demethylation pathways lies in the confirmation of bis(methylmercuric) sulphide or bis(methylmercuric)selenide, which are not stable in biological systems. Recently, the presence of bis(methylmercuric)selenide and dimethylmercury as intermediates in the in vitro degradation of methylmercury (seleno)amino acid complexes was confirmed by nuclear magnetic resonance (NMR) and gas chromatography−mass spectrometry (GC-MS), respectively. Based on the evidence of the presence of bis-(methylmercuric)selenide and dimethylmercury, Khan and Wang[12] proposed a mechanism for the degradation of methylmercury (seleno)amino acid complexes. However, the energetic feasibility for the degradation mechanism was not addressed. Based on computational approaches, we here report the thermodynamic feasibility of the degradation mechanisms of CH_3Hg-selenocysteinate as well as other possible alternative mechanisms for degradation. In order to better understand their similarity and/or dissimilarity, we also report the same for CH_3Hg-cysteinate. Since the basic mode of interaction of CH_3Hg with different amino and (seleno)amino acids (cysteine, glutathione, penicillamine, and methionine and their Se analogues) is the same,[21] we postulate that the same mechanisms should apply to other sulfur/(seleno)amino acids as well.

COMPUTATIONAL PROCEDURE

Calculations were performed with the Gaussian 03 (g03)[22] program suite and the Priroda code (version 6)[23−25] in the framework of the DFT.[26] The hybrid functional B3LYP[27,28] was employed for g03 calculations, and the generalized gradient approximation (GGA) functional due to Perdew, Burke, and Ernzerhof (PBE)[29] was employed for Priroda calculations. Three types of basis sets for different atoms have been used for the g03 calculations. The Stuttgart−Dresden basis set (SDD)[30] for the Hg atom, the 6-311+G(p) for the S and Se atoms, and

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the 6-31+G(p) basis for H, C, N, and O were used. To treat the (scalar) relativistic effects of the heavier atom, the SDD basis set for the Hg atom was used with the corresponding relativistic effective core potential. Priroda applies a scalar four-component relativistic method[31] with all electron basis sets. For all atoms, extensive correlation consistent triple-ζ polarized quality basis sets[24] for the large component, corresponding kinetically balanced basis sets for the small component, and appropriate auxiliary (fit) basis sets were employed. Further details of the computational protocol can be found elsewhere.[21] Frequency calculations have been performed in order to verify the nature of the stationary points and to calculate free energies. For each molecule studied in this article, we have obtained the true local energy minima. The solvation free energy has been evaluated at the gas-phase optimized geometries using the conductor-like polarizable continuum model (CPCM)[32] implemented in the g03 package. For test calculation on the solvation energy, we have applied the COSMO model[33−35] implemented in the ADF code.[36−39] The free energy calculated in the gas phase has been corrected by including the solvation free energy of each species. Unless otherwise stated, the energy presented in the following is the solvent corrected free energy. While Priroda and ADF calculations have been carried out as test calculations, the presented results are from g03 calculations unless otherwise stated. We have previously[21] shown that the all electron triple-ζ basis (p6) and the SDD/ECP basis sets for mercury (g03) resulted in very similar structural and thermochemical properties. A comparison of the reaction free energy using triple-ζ (p6) and SDD/ECP basis sets (g03) can be found in Table S1, Supporting Information.

RESULTS AND DISCUSSION

We shall start this section by recalling our earlier study[21] on the formation of CH_3Hg complexes with cysteine and selenocysteine. In that study, we had obtained structural features that were very close to the corresponding experimental values. In the energetic analysis, we had shown that the formation of CH_3Hg-selenocysteinate is thermodynamically favorable compared to its sulfur counterpart and that the Hg−S bond is stronger in CH_3Hg-cysteinate complexes than the Hg−Se bond in their Se analogues. Having found that the interactions of CH_3Hg with one amino or (seleno)amino acid are thermodynamically favorable, the question would arise whether binding more than one amino or (seleno)amino acid is possible, since there are experimental reports on these. For example, higher coordination for methylmercury was reported by Canty et al.,[40,41] Ghilardi et al.,[42−45] and Melnick et al.[46,47]

In order to investigate the possibility for the formation of Hg complexes with a coordination number higher than two (reactions 2 and 3), we have adopted the following model reactions for S and Se methyl mercury complexes:

$$CH_3HgOH + REH \rightarrow CH_3HgER + H_2O \quad (1)$$

$$CH_3HgOH + 2REH \rightarrow CH_3Hg(ER)_2^- + H^+ + H_2O \quad (2)$$

$$CH_3HgER + REH \rightarrow CH_3Hg(ER)_2^- + H^+ \quad (3)$$

Here, E = S (REH = cysteine) and Se (REH = selenocysteine), respectively. The model reactions are set up in such a way that they can best describe the experimental conditions.[12,48] Experimental conditions are usually too complex to properly model them in their entirety, and finding suitable computational model reactions is one of the challenges of applied quantum chemistry.[49]

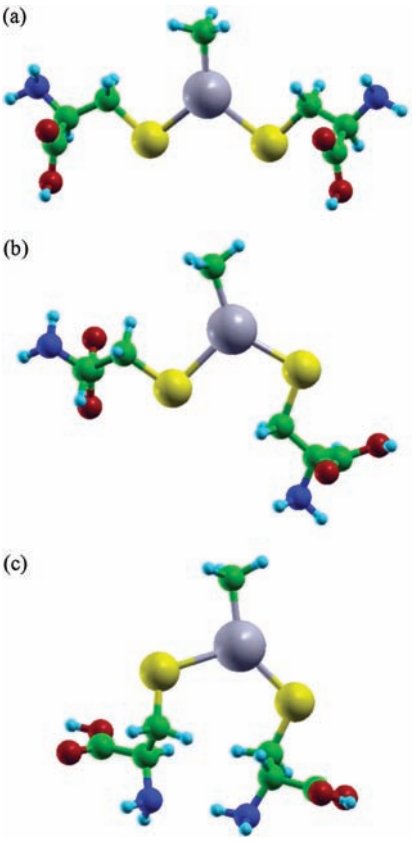

Figure 1. The optimized structures of three stable isomers [(a) isomer1, (b) isomer2, and (c) isomer3] of CH_3Hg diselenocysteinate. (The CH_3Hg dicysteinate has isomers with qualitatively similar structures.) The gray, yellow, red, blue, green, and light-blue balls represent Hg, Se, O, N, C, and H atoms, respectively.

We have studied reaction 1 in detail previously[21] and will now focus on the next set of reactions. CH_3Hg can directly react with two selenocysteine molecules (reaction 2), or after forming the CH_3Hg-selenocysteine complex, it can react with another selenocysteine molecule (reaction 3). In either cases, CH_3Hg diselenocysteinate is formed. Generally, in the experimental studies,[12,48] either amino or (seleno)amino acids is used as a reagent, and hence there was no opportunity to see whether a mixed complex with amino and (seleno)amino acids is formed. We have, however, also considered the mixed (both sulfur and seleno) amino complexes with CH_3Hg.

We have obtained three isomers for CH_3Hg diselenocysteinate. Figure 1 shows the optimized structures for three conformers of the diselenocysteine CH_3Hg complex. In Figure 1a, two cysteines are symmetrically bonded to the methylmercury group. On the other hand, in Figure 1c, two cysteine groups are asymmetrically bonded. Isomer2 (Figure 1b) is the intermediate structure between isomer1 (Figure 1a) and isomer3 (Figure 1c). We have observed qualitatively similar behavior for both, the S and Se containing complexes. The two Hg−Se (Hg−S) bond distances in the symmetric complexes are similar to each other (isomer1), whereas the two Hg−Se (Hg−S) bond distances are unequal in the asymmetric complexes (isomer2 and isomer3; see Table 1). In isomer3, the hydrogen atoms of the CH_2 groups

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Table 1. Hg-Linking Atom Bond Distance (Å) in the CH_3Hg Di(seleno)cysteinate

linking atom	Hg-linking atoms		linking atom	Hg-linking atom	
1st/2nd cysteine	Hg−S	Hg−S/O/N	1st/2nd seleno cysteine	Hg−Se	Hg−Se/O/N
S/S, isomer1	2.59	2.59	Se/Se, isomer1	2.69	2.69
S/S, isomer2	2.52	2.64	Se/Se, isomer2	2.69	2.76
S/S, isomer3	2.51	2.73	Se/Se, isomer3	2.63	2.79
S/O	2.45	2.77	Se/O	2.56	2.77
S/N	2.86	2.13	Se/N	2.94	2.14

Table 2. Free Energy (in kcal/mol) of CH_3Hg Di(seleno)cysteinate Complexes[a]

linking atom	CH_3Hg dicysteinate		linking atom	CH_3Hg diselenocysteinate	
1st/2nd cysteine	gas	solution	1st/2nd selenocysteine	gas	solution
S/S, isomer1	0.0	0.0	Se/Se, isomer1	0.0	0.0
S/S, isomer2	0.21	0.50	Se/Se, isomer2	0.29	0.10
S/S, isomer3	4.06	6.57	Se/Se, isomer3	2.14	4.14
S/O	6.54	2.45	Se/O	4.16	9.11
S/N	25.84	34.47	Se/N	28.84	37.10

[a] Relative to the most stable isomer in each case.

from two selenocysteine ligands and a hydrogen bonding between the amine groups of two (seleno)amino acids exert a repulsive and attractive force, respectively. This results in the two unequal Hg−Se (Hg−S) bond distances in this isomer. The Hg−Se (Hg−S) bond distance for isomer2 can be expected to be intermediate between those of isomer1 and isomer3, and we have indeed observed this (see Table 1). The Hg−S and Hg−Se bond distances in the mixed complexes are actually close to the corresponding values in their individual complexes.

We have also varied the linking atom of the second selenocysteine of the CH_3Hg complex. In the CH_3Hg diselenocysteine complex, we have used the N and O atoms for the linking to the Hg atom for the second (seleno)amino acids while keeping the S(Se) as linking atom to Hg for the first (seleno)amino acid. The relative stability of the latter complexes is, however, much lower than that of the respective S and Se bound complex. The relative stabilities both in the gas phase and in solution are presented in Table 2. The total energy of the mixed complex is in between those of the amino and (seleno)amino acid complexes. It is worth it to mention that we have obtained similar trends in the relative stability of different isomers using the COSMO solvation model.

From Table 2, it is clear that the symmetric CH_3Hg diselenocysteinate (isomer1) is the most stable isomer both in the gas phase and in solution. Although it is natural to think that the O and N atoms, being higher on the electronegativity scale, bind stronger with the metal atom, the observed bond distances, however, show otherwise (Table 1). The reason for this observation is that, in the O-bonded complexes, both oxygen atoms of the carboxylic acids approach to bind with the Hg atom. However, neither of them could bind to Hg strongly as this would require a very small O−Hg−O angle, which results in destabilization and a much higher energy. Therefore, the O-bonded (seleno)amino acids are weakly bonded. On the other hand, in the N-bonded complexes, the more electronegative N atom binds to the Hg atom strongly. This, however, leaves the (Se) S bonded (seleno)amino acids as weakly bonded. In our model reactions, one H atom of the NH_2 group is substituted by the Hg atom. Thereby, the N atom keeps its three bonding partners. In such a situation, the more electronegative N atom binds stronger with Hg than S and Se. However, depending on the pH of the reaction medium, the nature of the amine group varies, and it might not bind with the Hg. This is why in all experiments related to the (seleno)amino acid complexes with CH_3Hg, the dominant complexes are those with S (Se) bonded to Hg. These observations can be rationalized from the bond distances between the Hg and all linking atoms, as shown in Table 1.

Previously,[21] we have determined that the formation (reaction 1) of the Se complex is thermodynamically more favorable than the formation of its S counterpart, even though the Hg−S bond is stronger than the Hg−Se bond. Currently, we have also determined the free energy of reaction for reactions 2 and 3. Since the symmetric (Se) S bonded complexes are the most stable, we will therefore only use their energies in the following.

The determination of the free energy of a solvated proton is a challenging task. Tawa et al.[50] determined the free energy of the solvated proton with different theoretical approaches and concluded that the best value is −268.52 kcal/mol. We have used this value for our study.

The free energies of reaction of reactions 2 and 3 show that these reactions are thermodynamically unfavorable for both the S and Se complexes. The values are shown in Table 3 (the corresponding K values are presented in Table S2, Supporting Information). This observation might relate to the fact that the electronic configuration of the Hg atom is to some degree of noble gas type. In the reaction 1, the 6s orbital of Hg takes part in bonding to form stable complexes. However, adding another negatively charged ligand places an extra electron pair in the corresponding antibonding molecular orbital and weakens the bonding connected to Hg (reaction 2). This is further confirmed from the reactions involving inorganic Hg and selenocysteine, as shown in Table 4. Reactions 10 and 11 having two-coordinated Hg complexes as reaction products are thermodynamically favorable with the Se complexes being more favorable. On the

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Table 3. Free Energy of Reaction for Different Steps in the Degradation of CH_3Hg (Seleno) Amino Acid Complexes (kcal/mol)

		complexes		
	reactions	S	Se	mixed
1	$CH_3HgOH + REH \rightarrow CH_3HgER + H_2O$	−12.65	−18.17	
2	$CH_3HgOH + 2REH \rightarrow CH_3Hg(ER)_2^- + H^+ + H_2O$	11.31	−0.49	
2a	$CH_3HgOH + RSH + RSeH \rightarrow CH_3Hg(SR)(SeR)^- + H^+ + H_2O$			6.93
3	$CH_3HgER + REH \rightarrow CH_3Hg(ER)_2^- + H^+$	23.95	+17.68	
3a	$CH_3HgSR + RSeH \rightarrow CH_3Hg(SR)(SeR)^- + H^+$			18.96
3b	$CH_3HgSeR + ReH \rightarrow CH_3Hg(SR)(SeR)^- + H^+$			18.20
4	$CH_3HgOH + CH_3HgER \rightarrow (CH_3Hg)_2E + R{-}OH$	−22.29	−26.07	
5	$CH_3HgER + CH_3HgER \rightarrow (CH_3Hg)_2E + R{-}E{-}R$	−12.61	−13.02	
5a	$CH_3HgSR + CH_3HgSeR \rightarrow (CH_3Hg)_2S + R{-}Se{-}R$			−9.23
5b	$CH_3HgSeR + CH_3HgSR \rightarrow (CH_3Hg)_2Se + R{-}S{-}R$			−14.40
6	$CH_3HgER + HER + CH_3HgER \rightarrow (CH_3Hg)_2E + R{-}E{-}E{-}R + R{-}H$	−17.34	−28.83	
6a	$CH_3HgSR + HSeR + CH_3HgSR \rightarrow (CH_3Hg)_2S + R{-}S\text{-}Se\text{-}R + R\text{-}H$			−24.99
6b	$CH_3HgSR + HSeR + CH_3HgSR \rightarrow (CH_3Hg)_2Se + R{-}S{-}S{-}R + R{-}H$			−26.65
6c	$CH_3HgSeR + HSR + CH_3HgSeR \rightarrow (CH_3Hg)_2S + R{-}Se{-}Se{-}R + R{-}H$			−19.52
6d	$CH_3HgSeR + HSR + CH_3HgSeR \rightarrow (CH_3Hg)_2Se + R{-}S{-}Se{-}R + R{-}H$			−23.25
6e	$CH_3HgSR + HSR + CH_3HgSeR \rightarrow (CH_3Hg)_2S + R{-}S{-}Se{-}R + R{-}H$			−19.47
6f	$CH_3HgSR + HSR + CH_3HgSeR \rightarrow (CH_3Hg)_2Se + R{-}S{-}S{-}R + R{-}H$			−21.23
6 g	$CH_3HgSR + HSeR + CH_3HgSeR \rightarrow (CH_3Hg)_2S + R{-}Se{-}Se{-}R + R{-}H$			−25.04
6 h	$CH_3HgSR + HSeR + CH_3HgSeR \rightarrow (CH_3Hg)_2Se + R{-}S{-}Se{-}R + R{-}H$			−28.77
7	$(CH_3Hg)_2E \rightarrow (CH_3)_2Hg + HgE$	−23.40	−24.83	
8	$HgE(g) \rightarrow HgE(s)$		−64.35	
9	$(CH_3)_2Hg + H_2O \rightarrow CH_3HgOH + CH_4(g)$	−10.85	−10.85	

Table 4. Reaction Free Energy for the Interaction of Inorganic Mercury with Amino and Selenoamino Acid Complexes (kcal/mol)

		complexes containing	
	reactions	S	Se
10	$Hg(OH)_2 + HER \rightarrow Hg(OH)ER + H_2O$	−9.74	−16.63
11	$Hg(OH)ER + HER \rightarrow Hg(ER)_2 + H_2O$	−17.89	−25.88
12	$Hg(ER)_2 + HER \rightarrow Hg(ER)_3^- + H^+$	+40.20	+35.67
13	$Hg(OH)_2 + 3HER \rightarrow Hg(ER)_3^- + 2H_2O + H^+$	+12.57	+6.84

other hand, reactions 12 and 13 having three-coordinated Hg complexes as the reaction products are thermodynamically unfavorable, keeping a similar thermodynamic favorability of the Se complexes. Therefore, from the structural and energetic data we may conclude that CH_3Hg binds to only one (seleno)amino acid.

Before entering into a discussion of the details of the energetics involved in the degradation mechanism, let us revisit various experimental observations regarding the degradation of CH_3Hg (seleno)amino acid complexes. Of all the experimental reports, whether on biological systems or laboratory studies, the end products of the CH_3Hg (seleno)amino acid degradation are insoluble HgS and HgSe nanoparticles.[11,12,15,17,18,51,52] While the mechanism for this formation of insoluble nanoparticles is not clear, the formation of intermediate $(CH_3Hg)_2S$ (BMS) and $(CH_3Hg)_2Se$ (BMSe) is evident from various physiological and laboratory experiments.[12,17,18,51] Recently, Khan and Wang[12] provided NMR evidence for BMSe and GC-MS evidence for dimethylmercury in the demethylation of MeHg by (seleno)-aminoacids. They proposed a demethylation mechanism, where the BMSe is decomposed to $(CH_3)_2Hg$, which in turn is decomposed to CH_3HgOH. Tsai et al.[53] and Mounicou et al.[54] reported the formation of diselenide from (seleno)amino acids, among other potential intermediates. None of the experimental studies, however, reported any details on the thermodynamic feasibility of such degradation pathways.

Based on the above experimental observations, in particular the proposal of Khan and Wang,[12] we propose a set of intermediate reactions for the degradation of CH_3Hg (seleno)amino acid complexes, which leads to the formation of end products of HgS(s) and HgSe(s), respectively, while regenerating the CH_3HgOH in the processes. The free energy of reaction for the corresponding reactions is tabulated in Table 3. In the previous section, we have already discussed that the formation of CH_3Hg complexes with more than one (seleno)amino acid is unlikely. Therefore, it is more likely that the CH_3Hg (seleno)amino acid complexes decompose directly, which leads to the formation of HgS(s) [or HgSe(s)]. The formation of BMSe/BMS can occur in three different possible ways (reactions 4, 5, or 6). One way is the interaction of the already formed

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CH_3Hg (seleno)amino acid complex with another CH_3HgOH molecule (reaction 4). The second pathway is the intermolecular interaction of two CH_3Hg (seleno)amino acid complexes (reaction 5). The last pathway proceeds via the interaction of two CH_3Hg (seleno)amino acid complexes with another (seleno)amino acid molecule (reaction 6).

Among the three possibilities, the interaction of CH_3Hg (seleno)amino acid complexes with either CH_3HgOH or another (seleno)amino acid molecule is thermodynamically preferable. In addition to this thermodynamic feasibility, kinetic factors might play an important role in determining the degradation path. One of the two thermodynamically favorable paths (reaction 6) proceeds through the intermediate formation of a diselenide. The formation of BMSe with diselenide is in agreement with the observations of Khan and Wang.[12] It also supports the observation of intermediate products of diselenide by Tsai et al.[53] and Mounicou et al.[54] However, given the close values of the free energies of reactions 4 and 6, it is very difficult to conclude that the BMSe forms through only one pathway. Rather, in the presence of CH_3Hg in biological or any other systems containing amino or (seleno)amino acids, a number of chemical processes involving the formation and degradation of CH_3Hg (seleno)amino acid complexes will compete with each other. In addition, the kinetic control in a living cell could be quite different than in any pure aqueous solution and might play the deciding role with regards to BMSe/BMS formation.

Since both amino and (seleno)amino acids are present in cells (as free amino acids), it would be worthwhile to also consider the interactions (mixed reactions) between cysteine, selenocysteine, and CH_3Hg. The model reactions for mixed interactions are shown in Table 3. The reaction free energies for mixed reactions (reactions 2a, 3a, and 3b) have, in general, free energies that are in between those of the separate S- and Se-containing reactions. In the reaction pairs 5a/5b, 6a/6b, 6c/6d, 6e/6f and 6g/6h, for the same set of reactants, there are two possible products in each case. Although reactions 5a, 5b, and 6a through 6h are all thermodynamically favorable (like their counterparts reactions 5 and 6), the reactions having BMSe as a product are more favorable than the other reactions of each pair. This might hint at the possible role of selenium in the Hg−Se antagonism.

We have modeled the reactions to best describe the experimental setup[11,12] where CH_3HgOH is used as a source of CH_3Hg. However, in the real cell or aquatic environment, CH_3HgCl is present and interacts with amino and (seleno)amino acids. We have, therefore, performed test calculations on the interactions of amino acids with CH_3HgCl. Due to the higher formation constant values between Hg(II) and chloride, we obtained a positive free energy for complex formation (equivalent to reaction 1) and a more favorable free energy value for CH3HgCl formation (reaction 9), see Table S3, Supporting Information. However, we have observed the same trend between S- and Se-containing complexes as for hydroxide.

In order to check the relative favorability, we have further calculated the gas-phase enthalpy of formation for reactions involving S and Se separately (see Table S4, Supporting Information). In those reactions, we have observed that the Se-containing reactions are more exothermic than their S analogues.

Earlier, it was postulated[2] that the CH_3Hg-L-cysteinate is a molecular mimic of methionine. Due to this mimicry, CH_3Hg could easily cross the blood−brain barrier. We have shown in this study that the thermodynamic feasibility for the degradation of CH_3Hg-L-cysteinate is better than for its formation, which means there might not be available CH_3Hg-L-cysteinate to cross the blood−brain barrier. This finding suggests the possibility of having an alternative mechanism for crossing of the blood−brain barrier for CH_3Hg. Indeed, this hypothesis is well supported by the study of Hoffmeyer and co-workers.[55] They have studied the molecular mimicry between CH_3Hg-L-cysteinate and methionine. Based on electron density and structural features, they have demonstrated that there is no molecular-wide mimicry between CH_3Hg-L-cysteinate and methionine.

Although the formation of BMS and BMSe as an intermediate is evident from different experimental studies, the products from the degradation of those are not quite clear. Only recently, Khan and Wang[12] reported the formation of $(CH_3)_2Hg$ as a decomposition product of BMSe. In the process, solid HgSe precipitates as nanoparticles, depending, of course, on the amount of CH_3Hg present in the systems.[12] The proposed decomposition mechanism is, in fact, thermodynamically favorable, as we show in reaction 7. The computed free energies of reaction for S and Se are very close to each other. In calculating the reaction free energies of reaction 7, we have assumed a monomer of HgS and HgSe in solution. There is, however, experimental evidence[12,14,15] for the formation of HgS and HgSe nanoparticles in the degradation of CH_3Hg amino and (seleno)amino acid complexes. It is well-known that HgS and HgSe in the solid state are much more stable than in the gas phase. Von Szentpály[56] calculated the sublimation enthalpy for a series of crystals. For the HgS and HgSe crystals, he reported values of the sublimation enthalpy of 81.98 and 75.05 kcal/mol, respectively. Therefore, it can safely be assumed that the crystallization energy of HgS and HgSe from the solution will be energetically favorable. Moreover, we have calculated the cohesive energy of HgSe in the cubic crystal. Using the experimental unit cell dimension,[57] we have obtained 64.35 kcal/mol in energy[58] (reaction 8), which further confirms the energetic favorability of solidification of HgSe (and, by proxy, HgS). The findings of thermodynamic feasibility of HgSe precipitation in aqueous media may explain the observation of HgSe(s) granules in the liver of marine mammals, where CH_3Hg is induced through dietary uptake.[59,60] It also might suggest that sodium does not necessarily need to initiate the formation reaction of HgSe(s) as assumed in experiments.[16,19] Although there is no report on the level of concentration of (seleno)amino acids in natural waters, nanomolar to micromolar levels of (seleno)amino acids have been reported in surface water and sediments.[61,62] This presence of (seleno)amino acids might initiate the formation of HgSe(s) in the aquatic environment where CH_3Hg is present.

The $(CH_3)_2Hg$ formed in the previous step (reaction 7) reacts with a water molecule and forms CH_3HgOH, which completes the cycle (reaction 9). At first glance, it would appear that the formation of CH_3HgOH in reaction 9 would continue the CH_3Hg toxicity cycle. However, by balancing the reactions of the whole cycle (reactions 1, 4/6, 7, and 9) we can easily see that for every two molecules of CH_3HgOH, we have one molecule of HgSe which precipitates out from the cycle (see Figure 2). By removing one unit of Hg from solution, Se thus antagonized the toxicity of Hg in the biological systems.

Lastly, given the thermodynamic feasibility of the formation and degradation of CH_3Hg (seleno)amino acid complexes and not having any mimicry with methionine, it is very difficult to conclusively make predictions regarding the species that cross the blood−brain barrier. The stability of different intermediates might vary depending on the physiological conditions.

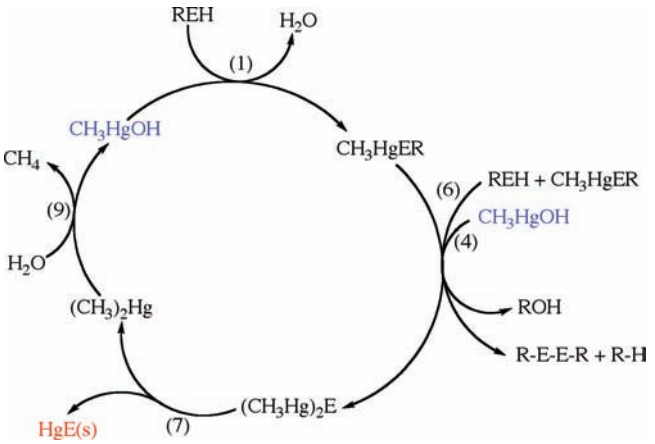

Figure 2. The cycle of the interactions of CH_3Hg with cysteine (REH, E = S) and selenocysteine (REH, E = Se). The numbers correspond to the reaction numbers of Table 3.

Depending on the physiological conditions and other related physiological phenomena, the blood−brain barrier crossing might be due to other species than CH_3Hg-L-cystinate. Therefore, it is necessary to revisit the hypothesis regarding the blood−brain crossing species.

Comparing the reaction free energies between analogous Se- and S-containing reactions, each and every Se-containing reaction is thermodynamically more favorable. This observation is in full agreement with our earlier studies. The reason for these observations is the relative binding strength involving Hg, S/Se, and C. Relatively weaker Hg−Se and Se−C bonded reactants will be thermodynamically favorable for a reaction compared to similar reactants containing stronger Hg−S and S−C bonds. This has been discussed in detail in our previous study.[21]

The calculated energetic data reveal that detoxification of mercury is thermodynamically favorable by both amino and (seleno)amino acids. However, overall thermodynamic feasibility for (seleno)amino acid complex is better than its sulfur counterpart. The preference of detoxification of mercury would thus be by (seleno)amino acids, which is in agreement with the experimental view on the role of selenium in the mammalian body. Our calculations are based on free cysteine and selenocysteine. Therefore, our proposed mechanism and other observations should be applied primarily to the free cysteine and selenocysteine that are present in the mammalian body.

CONCLUSION

In summary, a computational study has been carried out on the thermodynamics of the degradation mechanism[12] of CH_3Hg-selenocysteinate and its cysteine analogue. The formation of CH_3Hg-selenocysteinate is thermodynamically favorable. However, the interaction of CH_3Hg with two selenocysteine molecules (to form CH_3Hg-diselenocysteinate) is thermodynamically unfavorable. Instead, the CH_3Hg-selenocysteinate interacts with another CH_3Hg or another selenocysteine and decomposes to BMSe. The latter process is thermodynamically most favorable. BMSe in turn decomposes to $(CH_3)_2Hg$ and HgSe(s), which leaves the system by precipitation. $(CH_3)_2Hg$ then reacts with a water molecule and regenerates CH_3HgOH, completing the cycle. In the completed cycle, one molecule of HgSe is precipitated out from two molecules of CH_3HgOH. In this manner, Se antagonizes the toxicity of Hg.

The structural features of amino acid complexes are very much similar to those of (seleno)amino acid complexes. However, in every set of reactions in the whole degradation process, the Se-containing reactions are thermodynamically more favorable than their S-containing counterparts. Such thermodynamic favorability of (seleno)amino acid complexes might be responsible for the Hg−Se antagonism, despite having a similar degradation mechanism.

The degradation of CH_3Hg selenocysteinate and cysteinate is thermodynamically more favorable than their formation from CH_3Hg and selenocysteine/cysteine. This observation along with an earlier report on not having any molecular mimicry of CH_3Hg-L-cysteinate with methionine hints at the possibility of alterative species for crossing the blood−brain barrier.

ASSOCIATED CONTENT

Supporting Information. Full citation of ref 22 and Tables S1−S4. This material is available free of charge via the Internet at http://pubs.acs.org.

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