Theoretical Studies of Complexes between Hg (II) Ions and L-Cysteinate Amino Acids

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In this study, *ab initio* and density functional theory methods have been used to understand the structures and thermodynamic stabilities of complexes formed between L-cysteine and mercury (II) ions in neutral aqueous solution. To better understand the interaction between sulfur and mercury (II) ion, the MP2, B3LYP, M06-2X, and TPSS methods have been used to optimize $[HgSH_x]^{2-x}$, x = 1–4, complexes and compared to benchmark QCISD(T) structures. Furthermore, energies from these same methods are compared to CCSD(T)/CBS(2,3) energies. From these benchmark calculations, the M06-2X method was selected to optimize L-cysteinate-Hg(II) complexes and the MP2 method for estimating complex energies. L-cysteinate-mercury (II) ion complexes are formed primarily by forming a bond between cysteinate sulfur and the mercury ion. Stable complexes of L-cysteinate and mercury can be formed in 1:1, 2:1, 3:1, and 4:1 ratios. Each complex is stabilized further by interaction between carboxylate oxygen and mercury as well as hydrogen bonding among complex cysteinate ligands. The results indicate that at high cysteinate to Hg(II) ratios high-coordinate complexes can be present but at lower ratios the 2:1 complex should be dominant. © 2013 Wiley Periodicals, Inc.

DOI: 10.1002/qua.24565

Introduction

Mercury is a toxic heavy metal that can accumulate in the body and cause a variety of health problems. This toxic metal commonly exists in three forms: elemental, organic compounds, and inorganic compounds. Once mercury enters the body, it can begin to disrupt cellular functions in organs including the kidneys and brain.[1] Today, the most widely used treatment for mercury poisoning (hydrargyria) in humans involves chelation therapy. This treatment uses organic compounds that bond to heavy metal ions to facilitate excretion from the body. Currently used chelating agents include: 2, 3-dimercapto-1-propanesulfonic acid (DMPS), meso 2,3-dimercaptosuccinic acid (DMSA), ethylenediaminetetraacetic acid, and cysteine (Cys) with *N*-acetyl-cysteine (NAC). Studies have shown that treating mercury poisoning with DMPS or DMSA does remove some of the mercury; however, they have been proven ineffective at removing the metal from the brain.[2,3] Additional studies conducted on these two chelators have also shown that they are not optimized for the chelation of mercury, but are better suited for removing essential minerals such as zinc and copper.[4] In the case of treatment with cysteine in combination with NAC, evidence indicates an increased concentration of mercury in the brain and other organs.[3] This increase is attributed to the resulting complex mimicking amino acids that can move freely across cellular membranes by way of amino acid transporters.[5] With the current treatments of hydrargyria being less than optimal, further research needs to be conducted to identify more ideal Hg (II) chelators.

Molecules with thiol groups are known to have an affinity for mercury, thus it is often a good starting point for the design of mercury chelators. This idea is illustrated by Pearson's hard and soft acids and bases theory, which can aid in the explanation of the stabilities of metal-containing complexes.[6] This theory adds a further division of Lewis acids and bases by separating them into hard, soft, or borderline categories. Hard acids and bases usually are smaller in radius with a nonpolarizable character, whereas the soft acids and bases generally have a larger radius and a polarizable character. Utilizing this theory, stable complexes can be formed using the soft acid character of the mercury (II) ions and the soft base character of the sulfur atom. A number of experiments have observed that mercury (II) ions can embrace one of three different coordination domains: linear $Hg(SR)_2$, trigonal planar $Hg(SR)_3$, and tetrahedral $Hg(SR)_4$. At physiological pH values, ^{13}C and ^{1}H NMR studies have indicated that two cysteine thiolate groups with a linear S—Hg—S configuration is preferred but three-coordinate complexes are also present at high cysteine concentrations.[7] X-ray absorption fine-structure spectroscopy, in addition to, ^{199}Hg NMR, and Raman spectra on mercury (II)-cysteine complexes in basic solution by Jalilehvand et al. suggests that the thiol group on a cysteine ligand can form coordinated complexes that include from two to four cysteine ligands, which is mainly dependent on the activity of free cysteine.[8] ^{13}C NMR studies also indicate three-ligand coordination under high ligand to Hg(II) ratios.[9] An analysis of the S—Hg bond lengths for $Hg(SR)_2$, $Hg(SR)_3$, and $Hg(SR)_4$

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Contract grant sponsor: The National Science Foundation (NSF) (RUI grant); contract grant number: CHE 1011859.
Contract grant sponsor: Winston-Salem State University (NSF HBCU-Undergraduate Program) (J.W. and E.H.).

complexes in the Cambridge Structural Database found that the average bond lengths are 2.345, 2.446, and 2.566 Å, respectively.[10]

The thermodynamic parameters of mercuric ion complexation with cysteine containing dipeptide and tripeptide has been studied using isothermal titration calorimetry (ITC).[11,12] Each of these studies finds evidence for the formation of two types of complexes whose formation is dependent on the ratio of peptide to Hg(II) in solution. Computational studies have been performed to understand the structural and thermodynamics of cysteine-mercury complex formation. Mori et al. used density functional theory (DFT) calculations to understand 1:1 complexes of cysteine to Hg(II) and highlight the importance of the carboxylate group of cysteine in stabilizing the complex whereby a bond between the deprotonated cysteine sulfur and Hg(II) is formed.[13] Gas-phase DFT studies of neutral cysteine with Hg(II) ions also indicate the importance of carbonyl oxygen atoms in stabilizing the complex.[14] George et al. have used DFT to analyze the preferred geometric parameters about the Hg(II) ion in complexes with two and three ligands.[15,16] These studies predict that for the two-ligand complex an S—Hg—S angle is linear (~180 degrees), whereas the R—S---S—R dihedral angle (rotation about the linear S—Hg—S segment) is optimal at ~90 degrees, and the three-ligand complex adopts a trigonal planar structure about the Hg center. We have not found any studies in which a computational approach has examined the structures and thermodynamics of complexes formed when 1, 2, 3, and 4 cysteinate ($(NH_3)^+CH(CH_2S^-)CO_2^-$) ions are complexed with one Hg(II) ion in solution under biological pH (7.4).

In this study, appropriate quantum chemical methods are identified that will yield quality structures and energies for complexation of cysteine containing peptides with Hg(II) ions (Hg^{2+}). This requires an accurate description of the S—Hg interaction/bonding. To this end, gas-phase structures and energies of 1:1, 2:1, 3:1, and 4:1 complexes of HS^- with Hg(II) ions have been calculated by high level *ab initio* methods and compared with those from several lower level *ab initio* and DFT methods. Subsequently, solution phase structures and energies of 1:1, 2:1, 3:1, and 4:1 complexes of L-cysteinate ions with Hg(II) are explored to aid prediction of relevant complex ratios and structures that are present in aqueous solutions of biologic pH. These studies will serve as a basis for future studies of larger cysteine containing polypeptides and their complexes with Hg(II) ions.

Methodology

Thiolate-Hg(II) complexes

To determine an appropriate and efficient level of theory for the calculation of Hg(II) complexes structures with sulfur containing ligands, benchmark calculations for the complexes of Hg(II) with 1, 2, 3, and 4 thiolate ions (^-SH) ($[Hg(SH)_x]^{2-x}$, x = 1–4) were optimized using the quadratic configuration interaction including single and double substitutions with triples contribution QCISD(T)[17–21] method with a 6–311G(d,p) basis set for S and H and cc-pVTZ-PP[22] basis for Hg(II). The cc-pVTZ-PP basis set uses a relativistic effective core potential for the first 60 core electrons. The QCISD method is the best level of theory that we could carry out an optimization and frequency calculation with on the $[Hg(HS)_4]^{2-}$ complex; therefore, this structure will serve as the benchmark. The thiolate-Hg(II) complexes were also optimized via the MP2,[23] B3LYP,[24–27] M06-2X,[28,29] and TPSS[30] methods using a 6–31G(d) basis set for S and H and cc-pVDZ-PP basis for Hg^{2+} and compared to the benchmark QCISD(T) geometries. Vibrational frequency calculations were carried out at the same level as the optimization to ensure all structures are minima having no imaginary vibrational frequencies.

To generate benchmark energies for the complexes, single-point calculations were carried out with the coupled cluster method with single and double substitutions plus triple excitations included non-iteratively CCSD(T)/CBS(2,3)[31] level on the QCISD/6–311G(d,p)/cc-pVTZ-PP thiolate-Hg(II) structures. The energy of each structure is extrapolated to the complete basis set limit via Eqs. (1), (2), and (3).

$$E_{HF}^{\infty} = \frac{X^{\alpha}E_{HF}^{X} - Y^{\alpha}E_{HF}^{Y}}{X^{\alpha} - Y^{\alpha}} \quad (1)$$

$$E_{corr}^{\infty} = \frac{X^{\beta}E_{corr}^{X} - Y^{\beta}E_{corr}^{Y}}{X^{\beta} - Y^{\beta}} \quad (2)$$

$$E_{total}^{\infty} = E_{HF}^{\infty} + E_{corr}^{\infty} \quad (3)$$

where E_{HF}^{∞} is the infinite basis set energy excluding electron correlation (i.e., Hartree–Fock complete basis set limit) and E_{corr}^{∞} is the complete basis set limit for electron correlation. X and Y are the zeta quality of the two basis sets utilized (i.e., 2, 3 extrapolation utilizes double and triple zeta basis sets). Here, the double-zeta basis sets aug-cc-pVDZ-PP (Hg) and aug-cc-pVDZ (S and H) and the triple-zeta basis sets aug-cc-pVTZ-PP (Hg) and aug-cc-pVTZ (S and H) were used. The constants $\alpha = 3.4$ and $\beta = 2.4$ for a 2/3 extrapolation. All thiolate-Hg(II) calculations were performed using Gaussian09.[32]

L-Cysteinate-Hg(II) complexes

All L-cysteinate and L-cysteinate-Hg(II) complex geometries were optimized at the M06-2X/6–31G(d, p) level using a Stuttgart/Dresden (SDD)[33] basis set and pseudopotential, as implemented in Gaussian09, for the first 60 core electrons of mercury. This functional has recently been identified as providing good structures for small peptide molecules when compared with high-quality benchmark geometries.[34] It is chosen here because it provides a quality description of the thiolate-Hg(II) structures and they are designed to account for dispersion interactions, which are prevalent among peptide functional groups. Single-point energy calculations were carried out using the MP2 method considering all excitations with a 6–311G(d, p) basis set on nonmercury atoms and cc-pVDZ-PP basis set on mercury. The aqueous environment was accounted for by using the integral equation formalism model IEFPCM solvation model for M06-2X calculations and the COSMO solvation model for MP2 calculations. The size of

the van der Waals sphere for mercury in a complex structure was set to 2.34 Å when using the IEFPCM model, making it consistent with the conductor-like screening model COSMO model, whereas all other spheres used default values. To account for the energy of Hg(II) ions in solution, gas-phase energies were determined and the solvation free energy (ΔG_{solv}) from Rashin and Honig of −432.1 kcal/mol was added to obtain the energy in water.[35] Vibrational frequency calculations were carried out at the same level to ensure all structures are minima having no imaginary vibrational frequencies. In some instances, structures have small (<50 cm^{-1}) imaginary vibrational frequencies. M06-2X calculations were performed using Gaussian09, whereas MP2 calculations were performed using the ORCA 2.9 program.[36]

Results and Discussion

Thiolate-Hg(II) complex geometries

Bond lengths, bond angles, and torsion angles for 1:1, 2:1, 3:1, and 4:1 complexes of HS^- ions with Hg(II) calculated via the QCISD(T), MP2, B3LYP, M06-2X, and TPSS methods are provided in Table 1. To judge the quality of the structures, Table 1 also lists the mean unsigned error (M.U.E.) for the MP2, B3LYP, M06-2X, and TPSS geometric parameters relative to the benchmark QCISD(T) geometries. The M.U.E. of all complex bond lengths are listed separately from angles and torsions. For bond lengths, each method has fairly small M.U.E. ranging from 0.011 for the MP2 method to 0.044 for the B3LYP method. For bond angles and torsions, the M.U.E. ranges from 0.627 for the B3LYP method to 0.979 for the TPSS method. In each case, the MP2 method yields structures in excellent agreement to the QCISD(T) geometries. The DFT methods perform well; however, for bond lengths, the M06-2X method outperforms the others. Because bond potentials are steeper than bond angle and torsion potentials, higher level single-point energies on M06-2X geometries should be more similar to the single-point energies QCISD(T) structures. The M06-2X method is expected to be the more efficient DFT method for complexes containing larger peptides as it provided a good description of Hg—S bonds and is parameterized to treat nonbonding interactions. The M06-2X method will therefore be used for optimizations of cysteinate-Hg(II) complexes.

Several geometric parameters determined in this study can be compared to previously determined values. The gas-phase

Table 1. Geometric parameters (bond lengths, Å, angles, deg., and torsions, deg.) for complexes of HS^- ions with Hg(II) optimized using some ab initio and density functional theory methods.

Molecule	QCISD(T)[a]	MP2[b]	B3LYP[b]	M06-2X[b]	TPSS[b]
SH					
S—H	1.349	1.353	1.363	1.356	1.366
$[Hg(SH)]^{1+}$ C_s					
S—H	1.350	1.351	1.358	1.351	1.358
Hg—S	2.330	2.313	2.363	2.332	2.346
Hg—S—H	91.168	92.230	93.517	93.005	93.273
$Hg(SH)_2$ C_2					
S—H	1.344	1.345	1.353	1.345	1.354
Hg—S	2.327	2.308	2.363	2.346	2.356
Hg—S—H	93.087	94.530	94.800	94.793	94.296
S—Hg—S	178.794	178.380	178.253	178.636	177.972
H—S---S—H	89.361	89.153	88.970	90.231	89.486
$[Hg(SH)_3]^{1-}$ C_{3h}					
S—H	1.343	1.345	1.353	1.347	1.354
Hg—S	2.463	2.444	2.519	2.509	2.510
Hg—S—H	94.712	95.550	95.350	95.204	94.629
S—Hg—S	120.000	120.000	120.000	120.000	120.000
$[Hg(SH)_4]^{2-}$ [c]					
S—H	1.342	1.346	1.354	1.348	1.355
Hg—S	2.576	2.584	2.662	2.645	2.648
	2.598	2.585	2.680	2.666	2.671
	2.596	2.562	2.686	2.672	2.674
	2.620	2.611	2.711	2.699	2.702
Hg—S—H	91.200	91.410	91.068	89.663	89.698
	91.340	92.150	91.531	90.874	89.846
	91.630	92.420	91.733	90.902	90.040
	91.850	92.440	91.908	91.362	90.280
S—Hg—S					
Min[d]	105.580[c]	104.260	105.945	105.383	105.267
Max[d]	116.420[c]	115.760	115.383	117.211	117.359
M.U.E.[e]					
Avg. bond		0.011	0.044	0.032	0.038
Avg. angle/torsion		0.695	0.627	0.773	0.979

[a] Using 6–311G(d,p)/cc-pVTZ-PP basis sets. [b] Using 6–31G(d)/cc-pVDZ-PP basis sets. [c] Corresponds to QCISD geometry since a QCISD(T) optimization was not feasible. [d] Only the minimum and maximum values were extracted for comparison. [e] M.U.E is mean unsigned error relative to QCISD(T) or QCISD geometry.

$Hg(SH)_2$, $Hg(SH)_3$, and $Hg(SH)_4$ geometries (Table 1) provide Hg—S bond lengths in excellent agreement with the Cambridge Structural Database average bond lengths of 2.345, 2.446, and 2.566 Å, respectively.[10] Furthermore, the trend of increasing bond length with increasing number of ligands is replicated. The bond angles are also in good agreement with previous values of George et al.[15,16] providing S—Hg—S bond angles of ~178 degrees (almost linear) and H—S---S—H dihedral angles of ~90 degrees for two-ligand complexes and a trigonal planar geometry about the Hg in the three-ligand complex.

Thiolate-Hg(II) complex energies

Using the QCISD(T)/6–311G(d,p)/cc-pVTZ-PP optimized geometries, the complexation energies via single-point energy calculations from a number of *ab initio* and DFT methods were calculated and are provided in Table 2. CCSD(T)/CBS(2,3) complete basis set extrapolation values will serve as benchmark energies. Also, included in Table 2 are the M.U.E.s in energy for each of the four complex energies at each level of theory relative to the CCSD(T)/CBS(2,3) energies. The best performing levels are the MP2/aug-cc-pVDZ/aug-cc-pVDZ-PP, QCISD/6–311G(d,p)/cc-pVTZ-PP, and MP2/6–311G(d,p)/cc-pVDZ-PP levels with M.U.E values of 1.1, 1.5, and 2.1 kcal/mol, respectively. For the MP2 method, changing the basis from aug-cc-pVDZ/aug-cc-pVDZ-PP to aug-cc-pVTZ/aug-cc-pVTZ-PP causes the values to diverge, relative to the CBS values, resulting in M.U.E. of 7.8 kcal/mol. The CCSD(T) method with the aug-cc-pVTZ/aug-cc-pVTZ-PP underestimated the complexation energies to a small extent giving an M.U.E. of 3.4 kcal/mol. The B3LYP and M06-2X DFT methods with triple zeta quality basis sets perform near the bottom of the group with M.U.E. values of 7.3 and 14.9 kcal/mol, respectively. A good agreement by using a double zeta quality basis on Hg may be a result of tight electron density due to its 2+ charge. Furthermore, the MP2/6–311G(d,p)/cc-pVDZ-PP level performs very well for a relatively low level of theory. The excellent agreement may be fortuitous cancellation of errors given the commonly known oscillatory convergence of the nonvariational MP2 energies with increasing basis set. The MP2/6–311G(d,p)/cc-pVDZ-PP method has been chosen for calculating single-point energies on M06-2X geometries in further studies of peptide-Hg(II) complexes as these complexes are expected to be fairly large. The MP2 method will also better account for the energies associated with dispersion interactions, which are prevalent among peptide functional groups.

L-Cysteinate-Hg(II) complexes

To predict the favorable structures for complexes of L-cysteinate ion ($[cys]^-$) with mercury(II) in aqueous solution numerous complex structures in ratios of 1:1, 2:1, 3:1, and 4:1 ($Hg(II) + x[cys]^-$ to give $[Hg\text{-}cys_x]^{2-x}$, $x = 1–4$) were optimized and analyzed in terms of predicted stability. Figure 1 shows selected PCM-M06-2X/6–31G(d,p)/SDD structures for the $[Hg\text{-}cys_x]^{2-x}$, $x = 1–4$, complexes where the cysteinate sulfur is bonded with mercury. Note that Figure 1 does not provide an exhaustive compilation of complex structures. The selected structures have been chosen to highlight prominent features contributing to the stability of cysteine-Hg(II) complexes. Cysteine in neutral aqueous solution exists in a zwitterionic protonation state. The sulfur of the side chain is weakly acidic ($pK_a = 8.4$) so a small amount will exist in deprotonated form at pH = 7.4. Complexation of Hg(II) ions with cysteinate at the deprotonated sulfur will generate more deprotonated form to reestablish equilibrium according to Le Châtelier's principle; therefore, the deprotonated form should be readily available for complexation with mercury ions. Therefore, the complexes in Figure 1 are the result of zwitterionic cysteine anion, via deprotonation of the thiol group, complexing with Hg(II). Table 3 provides the raw molecular energies (E_e in hartree) for Hg(II), cysteine anion complexes shown in Figure 1 as well as the complexation energy (ΔE_e in kcal/mol) of each complex relative to Hg(II) and cysteinate ion at infinite separation.

For the 1:1 complexes (Fig. 1, structures 1a and 1b), the cysteinate ion can be a unidentate (bond with S) or bidentate (bond with S and interact with carbonyl O) ligand by complexing to Hg(II) ion. When cysteine anion binds in a unidentate fashion (1a), the complex is unstable having a complexation energy of +5.1 kcal/mol. The MP2 complexation energy for the bidentate complex (1b) is −14.6 kcal/mol, providing ~20 kcal/mol of stabilization. These energies indicate that a 1:1 complex is only stable in aqueous solution as a bidentate complex.

Table 2. Gas-phase complexation energies (E_e, kcal/mol) of HS^- and Hg(II) at several ab initio and density functional theory levels using QCISD(T)/6-311G(d,p)/cc-pVTZ-PP structures.

	HS^-:Hg^{2+} complex				
Single-point energy level	1:1	2:1	3:1	4:1	M.U.E.[a]
B3LYP/6-311++G(d,p)/aug-cc-pVTZ-PP	−436.0	−642.4	−679.8	−630.5	7.3
M06-2X/6-311++G(d,p)/aug-cc-pVTZ-PP	−422.2	−629.8	−671.3	−629.0	14.9
MP2/6-311G(d,p)/cc-pVDZ-PP	−430.2	−647.1	−691.4	−646.3	2.1
MP2/aug-cc-pVDZ/aug-cc-pVDZ-PP	−432.4	−646.6	−687.3	−641.6	1.1
MP2/aug-cc-pVTZ/aug-cc-pVTZ-PP	−438.4	−655.8	−697.3	−651.9	7.8
QCISD/6-311G(d,p)/cc-pVTZ-PP	−433.9	−647.4	−691.1	−645.8	1.5
CCSD(T)/aug-cc-pVDZ/aug-cc-pVDZ-PP	−427.1	−636.3	−677.2	−631.6	10.0
CCSD(T)/aug-cc-pVTZ/aug-cc-pVTZ-PP	−431.2	−643.1	−684.7	−639.4	3.4
CCSD(T)/CBS(2,3)	−433.0	−646.4	−688.8	−643.8	0.0

[a] M.U.E is mean unsigned error relative to CCSD(T)/CBS(2,3) values for the four complexes.

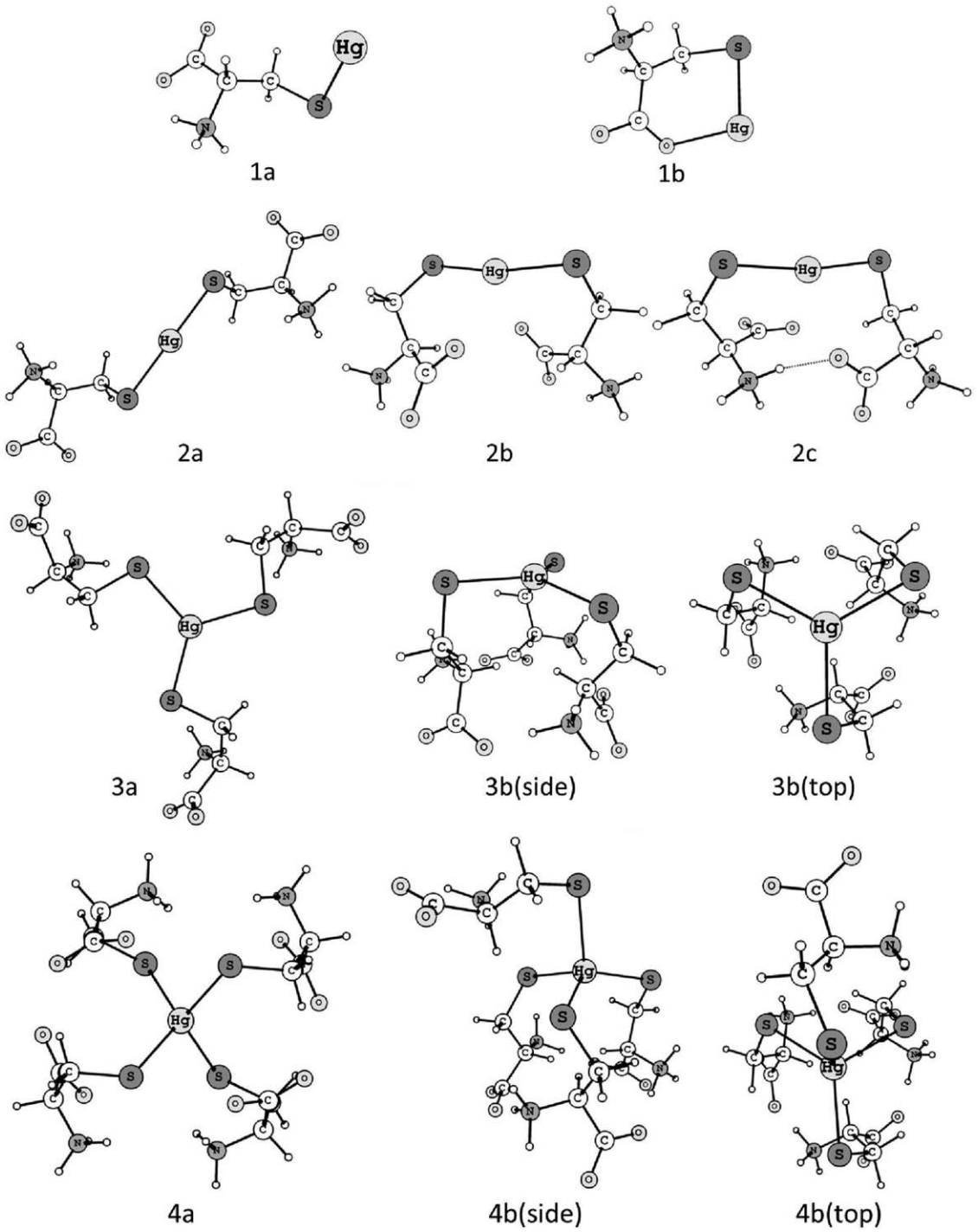

Figure 1. PCM-M06-2X/6–31G(d,p)/SDD structures for $[cys]^-$:Hg(II) complexes in ratios of 1:1 (1a-b), 2:1(2a–c), 3:1(3a–b) and 4:1(4a–b). Structures 3b and 4b are shown in top and side views.

Addition of a second cysteinate ion yields a 2:1 complex shown as structures 2a, 2b, and 2c in Figure 1. Structure 2a represents a complex in which both cysteinate ions act as unidentate ligands. This is a linear extended structure whereby the S—Hg—S bond angle is 179.5 degrees and the C—S---S—C dihedral angle is ~70 degrees while the cysteine ligands avoid interaction with each other. Formation of a second mercury-sulfur bond (1a to 2a) is over five times more stable than structure 1b with MP2 complexation energy of −82.3 kcal/mol. Rotation around each of the C—S bonds of complex 2a can give complex 2b. In complex 2b, the carboxylate groups are oriented so that a carboxylate oxygen from each cysteine is a bidentate ligand. The bidentate interaction causes the S—Hg—S angle to bow away from linear to 167.8 degrees and the C—S---S—C dihedral angle is reduced to ~65 degrees. At the MP2 level, this interaction provides ~11 kcal/mol of stabilization for 2b over 2a with a complexation energy of −94.1 kcal/mol. If, on the other hand, rearrangement occurs whereby the $-NH_3^+$ group of one

Table 3. Solution phase complexation energies (E_e, kcal/mol) of $[cys]^-$ + Hg(II) complex structures presented in Figure 1.

	M06-2X		MP2	
Molecule	E_e(PCM)	ΔE (complexation)	E_e(COSMO)	ΔE (complexation)
Cys-S^-	−721.3075584		−720.4143538	
Hg^{2+}	−153.04385[a]		−152.66277[a]	
1a	−874.3315199	12.5	−873.0686335	5.1
1b	−874.3670847	−9.8	−873.1001235	−14.6
2a	−1595.758327	−62.3	−1593.62190	−82.3
2b	−1595.787255	−80.5	−1593.640800	−94.1
2c	−1595.799581	−88.2	−1593.64930	−99.5
3a	−2317.094921	−80.6	−2314.068442	−102.7
3b	−2317.146632	−113.0	−2314.106746	−126.7
4a	−3038.437463	−102.5	−3034.483727	−103.5
4b	−3038.485547	−132.7	−3034.552894	−146.9

[a] SCF energy derived from gas phase energy plus −432.1 kcal/mol (−0.6886 hartree) ΔG(solvation) energy from Rashin and Honig.

cysteine can hydrogen bond with the carboxylate group of the other cysteine then complex 2c is formed. The strong hydrogen bonding interaction disrupts the bidentate interaction allowing the S—Hg—S bond angle to increase relative to 2b to 169.4 degrees and the C—S---S—C dihedral angle is reduced further to ∼38 degrees. Complex 2c has an MP2 complexation energy of −99.5 kcal/mol and is the most stable 2:1 complex isolated. The 2a–c series of complexation energies demonstrate the gain in complex stability that can be achieved via ligand interactions, with the greatest stability resulting from hydrogen bonding between the $-NH_3^+$ and carboxylate groups of two cysteine ligands. Furthermore, the bidentate complexation that includes interaction of mercury with a carboxylate oxygen (2b) also provides significant stabilization.

Two 3:1 cysteinate-Hg(II) complexes are shown in Figure 1 as 3a and 3b. Complex 3a is the unidentate analog of 1a and 2a resulting in a trigonal planar geometry about the central Hg atom and having C_3 symmetry. The cysteine groups are extended with no stabilizing nonbonding interactions among cysteine ligands. In contrast, complex 3b is formed by rotating the cysteine groups inward allowing for hydrogen bonding between the $-NH_3^+$ group of one cysteine with the carboxylate group of an adjacent cysteine. The trigonal planar center adjusts to a trigonal pyramidal center with a small (∼10 degrees) bow. The overall shape of complex 3b is that of a bowl with very close to C_3 symmetry. Hydrogen bonding among the cysteine groups gives significant stabilization to the 3:1 complex. At the MP2 level, the complexation energy for 3a is −102.7 kcal/mol and 3b is −126.7 kcal/mol, providing 24 kcal/mol of stabilization relative to 3a. In contrast to 2c, 3b is stabilized only by hydrogen bonding. The cysteine groups of the 3:1 complexes are able to adopt positions that maximize hydrogen bonding. This is seen by comparing the N—H---O distances in 2c (1.699 Å) to those observed in 3b (1.655 Å). To form the intercysteine hydrogen bonds, the intracysteine N—H---O hydrogen bonds are sacrificed lengthening from 1.705 Å in 3a to 2.064 Å in 3b.

Two 4:1 complexes are shown in Figure 1 as 4a and 4b. Complex 4a has a tetrahedral geometry around the mercury with the four-cysteine sulfur atoms bonded to the central Hg atom in a D_2 symmetric structure. Complex 4a is arranged to exclude stabilizing nonbonding interactions among the cysteine groups. Rotation of the cysteine groups allow for hydrogen bonding interactions among the cysteine groups to give complex 4b. Complex 4b is similar to 3b in that three of the cysteine groups hydrogen bond to yield a bowl-like structure and the fourth cysteine is bonded to the bottom of the bowl. The $-NH_3^+$ group on the fourth cysteine interacts with a sulfur on one of the other three cysteine groups. Hydrogen bonding gives 4b 43.4 kcal/mol of stability relative to 4a with complexation energies of −146.9 and −103.5 kcal/mol, respectively.

Each series of complex structures in Figure 1 follow a similar pattern whereby the structures 1a, 2a, 3a, and 4a are void of stabilizing, nonbonding interactions among the cysteine ligands and with mercury. The structures were chosen to compare the thermodynamic stability of 1:1, 2:1, 3:1, and 4:1 complexes offered by the S—Hg bond formation alone. The other structures show how intracomplex interactions between cysteine groups provide stability to a complex. We can compare the complexation energies of 1a, 2a, 3a, and 4a as each has a unidentate complexation with no stabilizing interactions between cysteine ligands or mercury ion. This comparison provides an indication of relative innate stability of each complexation ratio. The MP2 complexation energies of 1a, 2a, 3a, and 4a are +5.1, −82.3, −102.7, and −103.5 kcal/mol, respectively. The most significant gain in stability results from the transition from a 1:1 (1a) to a 2:1 (2a) complex with a stabilization of 87.4 kcal/mol. A further, smaller stability gain of 20.4 kcal/mol results by forming the 3:1 (3a) complex with a slight additional stability (0.8 kcal/mol) gained in forming the 4:1 (4a) complex. Except complex 1a, each ratio of cysteinate-Hg(II) yields a stable structure in aqueous solution.

Complexes 1b, 2c, 3b, and 4b are the lowest energy structures found for each cysteinate-Hg(II) complex ratio. Due to the flexibility of these complexes, there are many other possible structures but they have not been presented. We have confidence that the M06-2X method is providing quality complex geometries. Furthermore, a comparison of M06-2X and MP2 energies in Table 3 shows that the M06-2X trends in complexation energies are in good agreement with the MP2 values.

This study shows that sulfur containing peptide molecules are effective in binding to Hg(II) ions in aqueous solution and

gain additional stability from nonbonding interactions, especially hydrogen bonding, giving altered structures relative to gas- and solid-phase geometries. It is generally expected that Hg(II) prefers to form 2:1 ratio complexes. The data here are consistent with this expectation. If the MP2 energies in Table 3 are examined on a per Hg—S bond basis, the 1:1 complex stabilizes by 16.1 kcal/mol/bond, the 2:1 complex stabilizes by 42.0–49.9 kcal/mol/bond, the 3:1 complex stabilizes by 35.3–43.6 kcal/mol/bond, and the 4:1 complex by 26.5–37.4 kcal/mol/bond. Indeed, the 2:1 complexes have the highest per bond stabilization and should predominate in 2:1 solution concentrations. However, the significant per bond stabilization energies of the 3:1 and 4:1 complexes show that they should also be found when the solution contains a high concentration of cysteine relative to Hg(II). These data are also consistent with the observed multiple complex formations in ITC experiments. At higher cysteine to mercury (II) ratios, high coordinate complexes (3:1 or 4:1) may form and as the ratio moves toward 2:1 a transition to a 2:1 complex occurs.

Conclusions

In this study, *ab initio* and DFT methods have been used to understand the structures and thermodynamic stabilities of complexes formed between L-cysteine and mercury (II) ions in neutral aqueous solution. The MP2, B3LYP, M06-2X, and TPSS methods give a good description of the S—Hg bond structure when compared to benchmark QCISD(T) structures. Of these methods, only the MP2 method provided reliable complex energy values when compared to CCSD(T)/CBS energies. L-cysteinate and mercury ion complexes are formed primarily by forming a bond between a cysteinate sulfur and the mercury ion. Stable complexes of L-cysteinate and mercury can be formed in 1:1, 2:1, 3:1, and 4:1 ratios. Each complex is stabilized further by interaction between carboxylate groups and mercury as well as hydrogen bonding between complex cysteine ligands. A peptide molecule that is capable of maximizing each of the stabilizing interactions may serve as effective chelators for removal of mercury ions in humans.

Keywords: mercury (II) complexes • peptide complexes • cysteine • theoretical calculations • mercury poisoning

How to cite this article: J. Watts, E. Howell, J. K. Merle, *Int. J. Quantum Chem.* **2013**, DOI: 10.1002/qua.24565

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Received: 26 June 2013
Revised: 26 August 2013
Accepted: 15 September 2013
Published online on Wiley Online Library