Protection of Proton-Initiated Ligand Dissociation from Hg(II) Complexes with Bulky Cholyl Amide Arenethiolate by NH···S Hydrogen Bonding in an Aqueous Micellar Solution

Norikazu Ueyama,* Masahiro Inohara, Akira Onoda, Takafumi Ueno, Taka-aki Okamura, and Akira Nakamura

Department of Macromolecular Science, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan

Received September 15, 1998

Mercury(II) complexes which have a bulky cholyl amide group at the ortho or para position of benzenethiolate, $Hg[S\text{-}2\text{-}\{C_{23}H_{36}(OH)_3\}CONHC_6H_4]_2$ (**1**) with an intramolecular NH···S hydrogen bond and $Hg[S\text{-}4\text{-}\{C_{23}H_{36}(OH)_3\}CONHC_6H_4]_2$ (**2**), were synthesized to prepare an aqueous micellar solution. A hydrated Hg(II) ion was formed from the Hg(II) thiolate complexes, **1** and **2**, at the ligand dissociation point (pH 4.0 and 4.9, respectively) near the pK_a values (5.7 and 7.0, respectively) of the corresponding thiols. The hydrated Hg(II) ion was detected by the formation of Hg(0) species reduced with $Na_2S_2O_4$ in an aqueous micellar solution. The NH···S hydrogen bond lowers the pK_a value of the conjugated thiol to protect the Hg−S bond from dissociation by water under neutral conditions.

Introduction

The capture of Hg(II) ion with a thiolate ligand is an important detoxification process based on the inertness of the Hg(II) thiolate complexes under biological conditions in the field of environmental chemistry. The reduction of Hg(II) to Hg(0) is also a significant detoxification process in all biological systems.[1] Detoxification by the reduction of Hg(II) thiolate complexes, especially under physiological conditions, is considered to require a positively shifted redox potential of Hg(II)/Hg(I). The redox potential of Hg(II) thiolate complexes has not been established although a few papers have reported proposals for the reduction mechanism of Hg(II).[3] Apart from this, only one paper on the reduction of Hg(II) complexes in aqueous solution has been reported.[4]

Recently, we reported on the synthesis of various transition metal complexes with *o*-acylaminobenzenethiolate ligands, e.g., Mo(V),[5] Fe(II), Co(II),[6] and Cu(I).[7] These complexes possess an intramolecular NH···S hydrogen bond which contributes to the positive shift of their redox potentials. Similarly, the Hg(II) complexes of *o*-acylaminobenzenethiolate which have an intramolecular NH···S hydrogen bond were synthesized,[8] although these complexes are insoluble in water and in an aqueous micellar solution. In addition, they have a propensity to form an insoluble polymeric structure.[2]

There is another advantage to using *o*-acylaminobenzenethiolate in the introduction of a bulky acyl group on the acylamino group (which has an affinity with the long hydrocarbon media in micelles). Cholyl amide was attached to the arenethiolate as a large, hydrophobic acyl group at the ortho or para position to dissolve the Hg(II) complexes in an aqueous micellar solution. In the hydrocarbon media of this micelles, these hydrophobic environments enabled the formation of an NH···S hydrogen bond.

In order to investigate the stability of these Hg(II) thiolate complexes at different pH levels, an aqueous micellar system, which consists of two extremely different domains, is required. One is a hydrophobic domain, which supports the formation of the NH···S hydrogen bond, and the other is a hydrophilic domain to facilitate the proton exchange in an aqueous solution.

This paper presents the formation of a hydrated Hg(II) ion through the hydrolysis of the Hg(II) thiolate complexes at a low pH level. Then the Hg(II) ion in these complexes seems to be readily released in an aqueous micellar solution and reduced in the presence of a reductant, e.g., $Na_2S_2O_4$. The dissociation of the Hg(II) ion from the Hg(II) thiolate complexes occurs near the pK_a point of the thiol conjugated to the thiolate ligand. The shift of the pK_a value of the thiol by neighboring amide NH was also examined by at different pH levels.

Experimental Section

Materials. Cholic acid, isobutyl chloroformate, bis(2-aminophenyl) disulfide, tetraethylammonium borohydride, and triethylamine were of commercial grade. Tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF), and other solvents were purified by distillation before use. Triton X-100 and lauryl glucoside (LG) were of commercial grade.

Bis(2-cholylaminophenyl) Disulfide. Triethylamine (1.6 mL, 11 mmol) was added to a dry THF solution (100 mL) of cholic acid (4.5 g, 11 mmol), and then isobutyl chloroformate (0.56 mL, 4 mmol) was added at −15 °C. Immediately, the solution became cloudy. A dry THF solution (50 mL) of bis(2-aminophenyl) disulfide (0.5 g, 2 mmol) was added dropwise to the solution with vigorous stirring at −15 °C for 1 h and allowed to stand at room temperature overnight. When the solution was poured over ice and water, a pale yellow powder precipitated. The powder was dissolved in ethyl acetate (200 mL), and the ethyl acetate layer was successively washed with 2% aqueous HCl

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10.1021/ic9811042 CCC: $18.00
Published on Web 08/13/1999

solution, water, 4% aqueous $NaHCO_3$ solution, and water. The organic layer was dried over magnesium sulfate and concentrated under reduced pressure to give a pale yellow solid, which was recrystallized from acetonitrile. The pale yellow powder was dried over P_2O_5. Yield: 1.9 g (38%). ^{1}H NMR (dimethyl sulfoxide-d_6): δ 3.27 (s 2H), 3.62 (s 2H), 3.80 (s 2H), 3.97 (d 2H), 4.08 (d 2H), 4.28 (d 2H), 7.25 (m 6H), 7.55 (d 2H), 9.63 (s 2H). Anal. Calcd for $C_{60}H_{88}N_2O_8S_2 \cdot 3H_2O$: C, 66.51; H, 8.74; N, 2.59. Found: C, 66.69; H, 8.53; N, 2.69.

Bis(4-cholylaminophenyl) Disulfide. The compound was synthesized by the same method as described for bis(2-cholylaminophenyl) disulfide. The crude white solid was reprecipitated from acetone/hexane. The crude material was dried over P_2O_5. Yield: 0.75 g (23%). ^{1}H NMR (dimethyl sulfoxide-d_6): δ 3.18 (s 2H), 3.61 (s 2H), 3.79 (s 2H), 3.97 (d 2H), 4.08 (d 2H), 4.28 (d 2H), 7.43 (d 4H), 7.59 (d 4H), 9.98 (s 2H). Anal. Calcd for $C_{60}H_{88}N_2O_8S_2 \cdot 3H_2O$: C, 66.51; H, 8.74; N, 2.59. Found: C, 66.97; H, 8.85; N, 2.60.

Hg(S-2-cholyl-CONHC$_6$H$_4$)$_2$ (1). To a methanol solution (10 mL) of (S-2-cholyl-$CONHC_6H_4)_2$ (300 mg, 280 mmol) was added $NaBH_4$ (25 mg, 660 mmol), and the yellow solution changed to pale yellow. Then acetic acid (3 mL, 60 mmol) was added after a few minutes. After $HgCl_2$ (88 mg, 320 mmol) was added, the pale orange solution was concentrated and the residue was washed by a NaCl-saturated aqueous solution. A crude pale yellow powder was recrystallized from acetonitrile. The white powder was dried over P_2O_5. Yield: 0.2 g (43%). ^{1}H NMR (dimethyl sulfoxide-d_6): δ 3.18 (s 2H), 3.62 (s 2H), 3.80 (s 2H), 3.98 (d 2H), 4.08 (d 2H), 4.29 (d 2H), 6.89 (t 2H), 7.08 (t 2H), 7.47 (d 2H), 7.78 (d 2H), 9.06 (s 2H). Anal. Calcd for $C_{60}H_{88}O_8N_2S_2Hg \cdot 2H_2O$: C, 56.92; H, 7.32; N, 2.21. Found: C, 57.03; H, 7.21; N, 2.02.

Hg{S-4-cholyl-CONHC$_6$H$_4$}$_2$ (2). The complex was synthesized using the same method as described for **1**. The crude white powder was reprecipitated from ethyl acetate and dried over P_2O_5. Yield: 0.27 g (72%). ^{1}H NMR (dimethyl sulfoxide-d_6): δ 3.18 (s 2H), 3.62 (s 2H), 3.78 (s 2H), 3.98 (d 2H), 4.08 (d 2H), 4.28 (d 2H), 7.27 (d 4H), 7.38 (d 4H), 9.75 (s 2H). Anal. Calcd for $C_{60}H_{88}O_8N_2S_2Hg \cdot 4H_2O$: C, 55.34; H, 7.43; N, 2.15. Found: C, 55.29; H, 7.43; N, 1.96.

Hg(S-2-RCONHC$_6$H$_4$)$_2$ (R = CH$_3$, *t*-Bu). These complexes were synthesized using the same method as in the literature.[8]

Preparation of Aqueous Micellar Solutions. An equivalent volume (0.1 mL) of Triton X-100 or GL was added to a DMF solution (0.1 mL) of Hg(II) complex (22 mM) with stirring. Water (0.9 mL) was added to the 2 mM aqueous micellar solution. The micellar solutions of Hg(S-2-$RCONHC_6H_4)_2$ (R = cholyl) and Hg(S-4-$RCONHC_6H_4)_2$ (R = cholyl) are stable even for 10 days. The other Hg(II) complexes, Hg(S-2-$NHCORC_6H_4)_2$ (R = CH_3, *t*-Bu), were examined for micelle formation, but the aqueous micellar solutions of these complexes gradually crystallize to give heterogeneous solutions.

Samples for the measurement of ^{1}H NMR spectra were prepared from CD_3CN solutions (0.1 mL) of **1** and **2** by adding LG (0.1 mL) and water (0.5 mL).

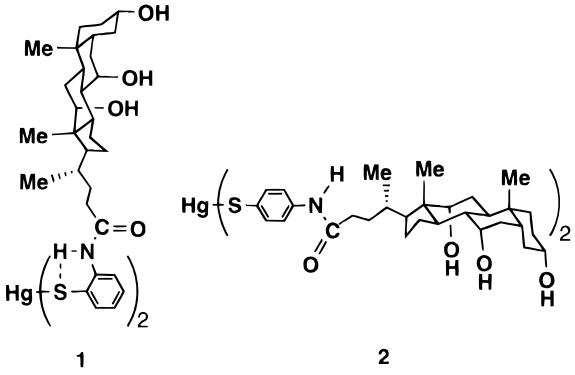
Reduction of Hg(II) Complex in Aqueous Micellar Solution. The pH of an aqueous micellar solution (2 mM, 0.3 mL) was adjusted in a quartz cell using a phosphate buffer. A large excess of $Na_2S_2O_4$ solution (1 M, 10 mL) was added, the mixture was stirred, and the quantity of reduced Hg(0) was determined by the turbidity, which was monitored at 800 nm absorption. The yield of Hg(0) was calculated on the basis of the yield of the authentic Hg(0) sample obtained by the reduction of $HgCl_2$. At the typical points, reduced Hg(0) was filtered off and the mercury vaporizer analysis of the solution was carried out to estimate the quantity of Hg(II) remaining.

Physical Measurements. An IR spectrum measurement was performed on a Jasco A-102 spectrometer and a Jasco DS-402G spectrometer. Samples were prepared as KBr pellets. ^{1}H NMR spectra were obtained with a JEOL EX-270 in dimethyl sulfoxide-d_6 at 30 °C. The dependence of the chemical shift of the amide NH signal on pH was measured in $CD_3CN/LG/H_2O$ = 1:1:5. ^{199}Hg NMR spectra were obtained with a JEOL GSX-400 in dimethylformamide (DMF) (20 mM, 30 °C). Dimethylmercury in DMF was used as an external reference. UV−visible spectra were measured on a Jasco Ubest-30 spectrometer. The pH of a thiol solution was determined using a Horiba pH-meter D-13 with a semimicro combination electrode 6069-10C and 6350-10D. The remaining Hg(II) was determined using a JEOL atomic absorption photometer with a mercury analyzer attachment.

Table 1. ^{199}Hg NMR Chemical Shifts of Hg[S-2-{$C_{23}H_{36}(OH)_3$}$CONHC_6H_4]_2$ (**1**) and Hg[S-4-{$C_{23}H_{36}(OH)_3$}$CONHC_6H_4]_2$ (**2**) in Acetonitrile

Hg(II) thiolate complexes	^{199}Hg chemical shifts
Hg[S-2-{$C_{23}H_{36}(OH)_3$}$CONHC_6H_4]_2$ (**1**)	−1185
Hg[S-4-{$C_{23}H_{36}(OH)_3$}$CONHC_6H_4]_2$ (**2**)	−891
Hg(S-2-*t*-Bu$CONHC_6H_4)_2$	−1142
Hg(S-2-$CH_3CONHC_6H_4)_2$	−1119
Hg(S-4-$CH_3CONHC_6H_4)_2$	−1072

Chart 1. Bulky Hg(II) Complexes



pK_a Determination. An aqueous micellar solution was prepared by the following procedure. An equivalent volume (0.3 mL) of Triton X-100 (Nacalai Tesque) was added to a DMF solution (0.3 mL) of thiol (∼15 mg, 3.0×10^{-5} mol) or thiolate (∼20 mg, 3.0×10^{-5} mol) with stirring. Water (2.7 mL) was added to the 10 mM aqueous micellar solution. Titrations were performed with 0.1 M NaOH or 0.1 M HCl aqueous solution (Nacalai Tesque) at room temperature. The pK_a value was estimated by the following equation:

$$pK_a = pH - \log[Na^+] + \log\{[thiol]_0 - [Na^+]\}$$

or

$$pK_a = pH + \log[Cl^-] - \log\{[thiolate]_0 - [Cl^-]\}$$

Results and Discussion

^{199}Hg NMR of Hg(II) Thiolate Complexes in DMF. Table 1 shows the chemical shifts in the ^{199}Hg NMR spectra of various Hg(II) thiolate complexes in DMF at room temperature. The detection of a ^{199}Hg NMR signal of an aqueous micellar solution of these Hg(II) thiolate complexes was unsuccessful. However, each of Hg[S-2-{$C_{23}H_{36}(OH)_3$}$CONHC_6H_4]_2$ (**1**) and Hg[S-4-{$C_{23}H_{36}(OH)_3$}$CONHC_6H_4]_2$ (**2**) (Chart 1) exhibits a ^{199}Hg signal at −1185 and −891 ppm, respectively, in DMF at 303 K. Since the Hammett σ_p constant is nearly zero for an acylamino group, the ^{199}Hg chemical shift is expected to be −1080 ppm as reported in the previous paper.[8] The upfield shift (Δ105 ppm) of the signal in **1** is due to the electron-withdrawing effect of the amide group at the ortho position, whereas the downfield shift of a ^{199}Hg NMR signal of **2** is due to the electron-donating effect.[9] The electron-withdrawing effect comes from the effect of the NH···S hydrogen bond as discussed in the previous paper.[8] The detection of any ^{199}Hg signal of these complexes in aqueous micellar solution was unsuccessful. This is because signal broadness with the small T_1 value was caused by high viscosity under these conditions.[10] The slow rotation

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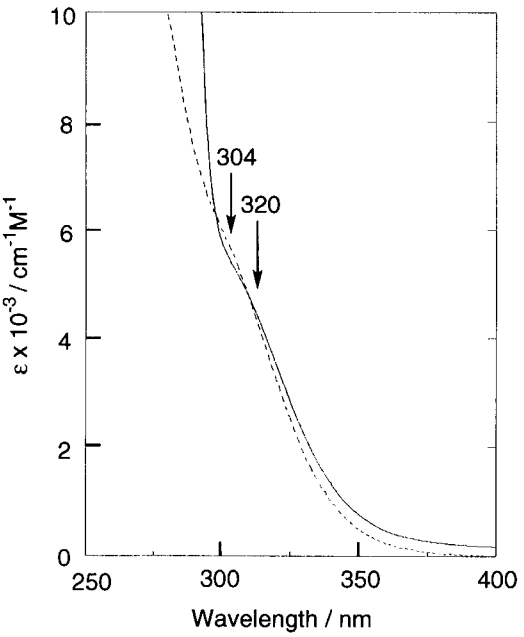


Figure 1. UV−visible spectra of Hg[S-2-{$C_{23}H_{36}(OH)_3$}$CONHC_6H_4$]$_2$ (**1**) in an aqueous micellar solution (—) and in MeOH (- - -) at room temperature.

of molecules in an aqueous micellar solution results in a large shift anisotropy. This leads to a short T_1 value.

Formation and Stability of Hg(II) Thiolate Complexes in Aqueous Micellar Solution. Thermal stability and its pH dependence in the aqueous micellar solution of Hg(S-2-$RCONHC_6H_4$)$_2$ (R = CH_3, *t*-Bu, $C_{23}H_{36}(OH)_3$) was examined. Thus, a solution of **1** and **2** maintains homogeneity for 48 h at room temperature. The other complexes give a white precipitate in a period ranging from 1 min to 24 h depending on the exact conditions. The large cholyl group in **1** interacts with the long hydrocarbon part of Triton X-100 micelles without crystallization. The low crystallinity contributes to the formation of clear micelles and provides a clear solution. Figure 1 shows the UV−visible spectra of **1** in an aqueous Triton X-100 micellar solution (acetonitrile/Triton X-100/H_2O = 1:1:5). A shoulder at 303 nm in **1** is assignable to the ligand-to-metal charge transfer band.[11]

The pH dependence of the thermal stability of **1** and **2** in an aqueous micellar solution was examined using the ^{1}H NMR spectroscopic method. Only a small change (0.03 ppm) in the chemical shift of the ^{1}H NMR amide NH signal was observed at various pHs in an aqueous micellar solution (CD_3CN/LG/H_2O = 1:1:5) at room temperature, although a broad inflection point was detected at ca. pH 3−4 for **1**. The small difference in the chemical shifts reflects a similar environment for the amide NH groups between **1** and protonated 2-cholyl-$CONHC_6H_4$-SH. This is ascribed to the presence of an extremely weak interaction between the amide NH and sulfur through the strongly covalent Hg−S bond.[8] Therefore, only a similar strong metal−sulfur interaction was reported for those of Cu(I), Mo(V), Fe(II), and Co(II) thiolate complexes.[5−7]

Reduction of Hg(II) Thiolate Complexes. Any clear redox couple for these Hg(II) thiolate complexes was not observed in the range −2.5 to +2.5 V (vs SCE) in DMF or acetonitrile using cyclic voltammetry, although a paper reported the reduction potentials for various Hg(II) complexes in an aqueous solution.[1] We conclude that Hg(II) thiolate complexes do not exhibit any redox couple in the above range because of their extremely negative redox potentials in an organic solvent. Therefore, our data suggest that hydrated Hg(II) ion formed by the dissociation of the Hg(II) thiolate complexes is readily reduced to Hg(0) as shown in Scheme 1.

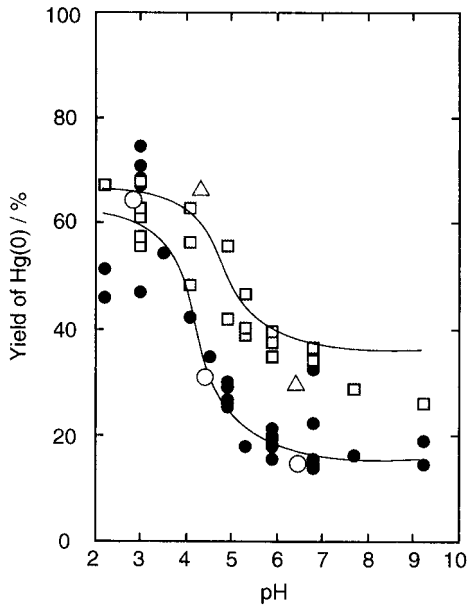
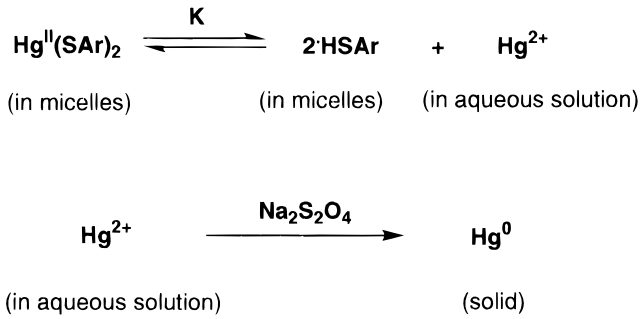


Figure 2. pH dependence of the quantity of Hg(0) generated by the $Na_2S_2O_4$ reduction of Hg[S-2-{$C_{23}H_{36}(OH)_3$}$CONHC_6H_4$]$_2$ (**1**) and Hg-[S-4-{$C_{23}H_{36}(OH)_3$}$CONHC_6H_4$]$_2$ (**2**) in an aqueous micellar solution. Data from the UV−visible turbidity analysis are represented by (●) for **1** and by (□) for **2**. Data from the mercury vaporizer analysis are inserted with (O) for **1** and (△) for **2**. The solid line drawn through the data was obtained by the regression analysis for a single ideal species using a KaleidaGraph program.

Scheme 1. Reduction of Hg(II) to Hg(0) in Aqueous Micellar Solution



In the presence of a mild reductant, $Na_2S_2O_4$, the reduction to Hg(0) requires the formation of a hydrated Hg(II) species in an aqueous solution. The enhanced dissociation of the thiolate ligand is ascribed to its high pK_a value as the thiol. The Hg(0) was determined by measuring its turbidity at 800 nm using UV−visible absorption spectroscopy. Figure 2 shows the amounts of generated Hg(0) against various pH values in an aqueous micellar solution. Until the thiolate ligand dissociates from the Hg(II) complexes by hydrolysis, the reduction of Hg(II) will not proceed. The dissociation of the ligand in **1** occurs at a lower pH than for **2**. **1** exhibits an inflection point at pH 4.0. This value is close to the pK_a value (5.7) of the conjugated acid form, i.e., 2-{$C_{23}H_{36}(OH)_3$}$CONHC_6H_4SH$, in an aqueous Triton X-100 solution. This value corresponds to the pK_a 4.2 value

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determined by the chemical shift of the ^{1}H NMR amide NH signal in an aqueous LG micellar solution. Ligand dissociation is possible at a lower pH than the pK_a value of the thiol because the Hg−S bond is supported by a strong covalency. In contrast, **2** exhibits an inflection point at pH 4.9 since 4-{$C_{23}H_{36}(OH)_3$}-$CONHC_6H_4SH$ has a pK_a value of 7.0 in an aqueous micellar solution.

Mercury(II) complexes which have a bulky cholyl amide group at the ortho and para positions of benzenethiolate, Hg[S-2-{$C_{23}H_{36}(OH)_3$}$CONHC_6H_4]_2$ (**1**) and Hg[S-4-{$C_{23}H_{36}(OH)_3$}$CONHC_6H_4]_2$ (**2**), were synthesized to make an aqueous micellar solution. After a hydrated Hg(II) ion forms from Hg(II) thiolate complexes at the ligand dissociation point near the pK_a of the corresponding thiol, the reduction of the hydrated Hg(II) ion to Hg(0) occurs. The intramolecular NH···S hydrogen bond in **1** makes the pK_a value of thiol lower. Thus, the hydrogen bond protects the dissociation of the Hg−S bond under neutral conditions.

The lability of the Hg(II)−S bond has been studied on Hg(II) complexes with L-cysteine, glutathione, and D,L-penicillamine in an aqueous solution.[12−14] These complexes are thermodynamically stable in a neutral aqueous solution, but are accompanied by a fast Hg−S exchange. Although the lability of the Hg−S bond has been reported, the Hg−S bond is inert against reduction under neutral conditions until the micellar Hg(II) solution attains the protonation point of the thiolate ligand. Therefore, the lower shift of pK_a in a ligand thiol by the intramolecular NH···S hydrogen bond in **1** prevents the reduction of Hg(II).

IC9811042

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