Contribution from the Department of Chemistry,
University of Wisconsin—Milwaukee, Milwaukee, Wisconsin 53201

Competition of Thiols and Cyanide for Gold(I)

George Lewis and C. Frank Shaw III*

Received July 23, 1985

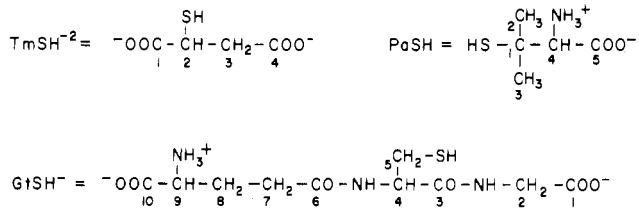
Thiol ligands are capable of partially displacing cyanide from $Au(CN)_2^-$, demonstrating that their affinity for gold(I) is very high. Raman and ^{13}C NMR evidence for the formation in solution of mixed-ligand complexes, $RSAuCN^-$, which disproportionate to $Au(CN)_2^-$ and $(RS)_2Au^-$, is presented. With use of literature values for reduction potentials, the conditional equilibrium formation constants at pH 7.0 of (cysteinato)gold(I), $K = 1.5 \times 10^{24}$, and bis(cysteinato)gold(I), $K = 3 \times 10^{28}$, were calculated for comparison to the formation constants of $Au(CN)_2^-$. A potential gold metabolite, bis(glutathionato)gold(I), was generated in situ, characterized by ^{13}C NMR, and studied in reactions with cyanide.

Introduction

Two factors prompted us to investigate the equilibrium competition of cyanide and thiols for gold(I). First, mono- and bis(thiolato)gold(I) species, AuSR and $Au(SR)_2^-$, are important as antiarthritic drugs,[1-3] as possible metabolites of drugs,[4,5] and as models for gold–protein complexes which form through bonds to sulfhydryl groups.[6,7] Chrysotherapy patients who are tobacco smokers accumulate into their red blood cells gold from injectable drugs, while nonsmokers do not.[8] Cyanide from the inhaled smoke alters the metabolism of gold.[9] Thus the reactions of cyanide with gold(I) thiolates in vivo are therapeutically significant. Second, syntheses of the 1:1 gold(I) thiolates often involve reduction of gold(III) by a thiol,[10] SO_2,[11] or $S(CH_2CH_2OH)_2$,[12] sometimes leading to undesirable side products. The report by McKinley, Brown, and Smith[13] that (L-cysteinato-*S*)gold(I) (AuSCy) can be prepared in good yield from $K[Au(CN)_2]$ via ligand exchange was attractive because the reaction might provide a clean and general synthesis of gold(I) thiolates.[14]

Mössbauer and EXAFS data have established that gold(I) thiolates such as those of thiomalate (TmSH), thioglucose (TgSH), cysteine (CySH), and gluthathione (GtSH) have AuS_2 coordination environments formed by bridging of the thiolate ligands between two gold(I) ions to form oligomers, $[AuSR]_n$.[15,16] The oligomers react with additional thiol to form simpler complexes formulated as $[Au(SR)_2^-]$[4,15,17,18] or $[Au(SR)_{1.75}]$;[5] the equilibrium constant for the reaction between (cysteinato)gold(I) and cysteine has been determined (RSH = cysteine; $K_1 = 2.1 \times 10^{-3}$).[4]

Chart I



$$(1/n)[AuSR]_n + RSH \overset{K_1}{\rightleftharpoons} Au(SR)_2^- + H^+ \quad (1)$$

The reaction is favorable at neutral to basic pHs ($K_{cond}^{7.4} = [Au(SR)_2^-]/[AuSR][RSH] = 4.4 \times 10^4$). Equilibrium constants for the formation of the 1:1 complex (reaction 2) have not been

$$Au^+ + RSH \overset{K_2}{\rightleftharpoons} (1/n)[AuSR]_n + H^+ \quad (2)$$

reported, although the chemical behavior of these species indicates that the oligomers are stable structures.[1] The formation constant of $Au(CN)_2^-$ is known to be very large (4×10^{28} or 10^{39}).[20,21] The equilibria between cyanide and thiolates for gold(I) may be complex since at least four different two-coordinate gold(I) species ($[AuSR]_n$, $Au(SR)_2^-$, $Au(CN)_2^-$, $Au(SR)CN^-$) may be formed by ligand-scrambling reactions. Formation of three-coordinate species, which are common with neutral ligands such as phosphines but not with thiolates,[2] would further complicate the system.

The structures, atom-numbering schemes, and abbreviations of the thiol ligands employed in this study are shown in Chart I.

Experimental Section

From Aldrich Chemical Co. (Milwaukee, WI) were obtained reduced glutathione, D-(−)-penticillamine, sodium aurothiomalate monohydrate, and mercaptosuccinic acid, from Sigma Chemical Co. (St. Louis, MO) *N*-acetyl-L-cysteine, L-cysteine hydrochloride monohydrate, L-cysteine methyl ester hydrochloride, and β-1-D-thioglucose sodium salt, and from Stohler Isotope Chemicals (Waltham, MA) 99% $K^{13}CN$. Dr. Nancy Schaffer-Memmel and Anne Hormann kindly provided the samples of gold(I) glutathione.[6] $K[Au(CN)_2]$ was prepared by a literature method.[22]

Infrared spectra (Nujol mulls) were obtained on a Nicolet MX1 spectrometer. Gold analyses were performed on an Instrument Laboratories 357 AA/AE spectrophotometer using serial dilutions of Spex Industries $K[Au(CN)_2]$ standards. Carbon-13 NMR data were acquired on a Bruker WP 250 multinuclear spectrometer and referenced against 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt (TSP). Raman spectra were excited with 488-nm Ar^+ excitation (200 mW) and accumulated with a Spex 1401 double monochromator interfaced to a IBM

* To whom correspondence should be addressed.

(1) Sadler, P. J. *Struct. Bonding (Berlin)* **1976**, *29*, 171-214.
(2) Shaw, C. F., III. *Inorg. Perspec. Biol. Med.* **1979**, *2*, 287-355.
(3) Brown, D. H.; Smith, W. E. *Chem. Soc. Rev.* **1980**, *9*, 217-240.
(4) Shaw, C. F., III; Schmitz, G.; Thompson, H. O.; Witkiewicz, P. *J. Inorg. Biochem.* **1979**, *10*, 317-333.
(5) Isab, A. A.; Sadler, P. J. *J. Chem. Soc., Dalton Trans.* **1982**, 135-140.
(6) Shaw, C. F., III; Schaeffer, N. A.; Elder, R. C.; Eidsness, M. K.; Trooster, J. M.; Calis, G. H. M. *J. Am. Chem. Soc.* **1984**, *106*, 3511-3521.
(7) Laib, J.; Shaw, C. F., III; Petering, D. H.; Eidsness, M. K.; Elder, R. C.; Garvey, J. S. *Biochemistry* **1985**, *24*, 1977-1986.
(8) Lewis, D.; Capell, H. A.; McNiel, C. J.; Iqbal, M. S.; Brown, D. H.; Smith, W. E. *Ann. Rheum. Dis.* **1983**, *42*, 566-570.
(9) Graham, G. G.; Haavisto, T. M.; Jones, H. M.; Champion, G. D. *Biochem. Pharmacol.* **1984**, *33*, 1257-1262.
(10) Shaw, C. F., III; Schmitz, G. P. *Inorg. Synth.* **1982**, *21*, 31-33.
(11) Shaw, C. F., III; Cancro, M. P.; Butler, I. S. *J. Labelled Compd. Radiopharm.* **1979**, *16*, 827-831.
(12) Hill, D. T.; Levison, S. H. *J. Labelled Compd. Radiopharm.* **1982**, *20*, 363-375.
(13) Brown, D. M.; McKinley, G. C.; Smith, W. E. *J. Chem. Soc., Dalton Trans.* **1978**, 199-201.
(14) Abbreviations: specific thiols (RSH) represented as $GtSH^-$ (glutathione), $TmSH^{2-}$ (thiomalate), H_2TmSH (thiomalic acid), CySH (cysteine), and PaSH (penicillamine); mono- and bis(thiolato)gold complexes represented as $[AuSR]_n$ or AuSR and $Au(SR)_2^-$, respectively; DD = double distilled, TSP = (trimethylsilyl)propionate, sodium salt.
(15) Hill, D. T.; Sutton, B. M.; Isab, A. A.; Razi, T.; Sadler, P. J.; Trooster, J. M.; Calis, G. H. M. *Inorg. Chem.* **1983**, *22*, 2936-2942.
(16) Elder, R. C.; Eidsness, M. K.; Heeg, M. J.; Tepperman, K. G.; Shaw, C. F., III; Schaeffer, N. *ACS Symp. Ser.* **1983**, *No. 209*, 385-400.
(17) Shaw, C. F., III; Eldridge, J.; Cancro, M. P. *J. Inorg. Biochem.* **1981**, *14*, 267-274.
(18) Danpure, C. J. *Biochem. Pharmacol.* **1976**, *25*, 2343.
(19) Isab, A. A.; Sadler, P. J. *J. Chem. Soc., Dalton Trans.* **1981**, 1657-1662.
(20) Bodlander, G. *Ber. Dtsch. Chem. Ges.* **1903**, *36*, 3933.
(21) Latimer, W. M. "Oxidation Potentials"; Prentice-Hall: New York, 1952; p 195.
(22) Brauer, G. D. "Handbook of Preparative Inorganic Chemistry", 2nd ed.; Academic Press: New York, 1965; Vol. II, p 1065.

0020-1669/86/1325-0058$01.50/0

PC Jr. microcomputer. The slit width was 5 cm^{-1}.

(L-Cysteinato)gold(I).[13] $K[Au(CN)_2]$ (0.3624 g, 1.26 mmol) in 5.0 mL of double-distilled (DD) H_2O and L-cysteine hydrochloride monohydrate, (22.74 g) in 95 mL of DD H_2O were mixed. After 3–4 min, the acidic solution (pH 1) became cloudy. The solution was stored at 4 °C overnight, and then the amorphous precipitate was collected and washed with ethanol and then anhydrous ether. Finally the pale yellow amorphous powder was dried under vacuum over P_2O_5: yield 0.353 g (88%); mp 175 °C dec. The IR spectrum displayed strong peaks for ν_{NH} (3160 cm^{-1}) $\nu_{C=O}$ (1645 cm^{-1}), and ν_{C-O} (1248 cm^{-1}). Anal. Calcd for $C_3H_6AuNO_3S$: C, 11.36; H, 1.91; N, 4.42; Au, 62.10. Found: C, 11.33; H, 1.39; N, 4.44; Au, 61.95.

Other Attempted $K[Au(CN)_2]$/RSH Reactions. $K[Au(CN)_2]$ (444 mg, 1.54 mmol) and GtSH (476 mg, 1.549 mmol) were independently dissolved in minimum volumes of DD H_2O and then mixed. When no precipitate formed after 24 h, the solvent was stripped. The IR spectrum of the resulting solid showed only unreacted starting materials to be present. Similar results were obtained with PaSH, TgSH, and ATgSH sometimes with use of mixed aqueous alcohol solutions. A gold(I) thiolate of *N*-AcCySH was precipitated from a water/ethanol solution, but satisfactory elemental analyses could not be obtained.

^{13}C NMR Spectra of RSH/$Au(CN)_2$ Mixtures. The spectrum of 0.2 M $K[Au(CN)_2]$ and 0.2 M GtSH dissolved in 80% H_2O/20% D_2O containing TSP was obtained. The reactions of $K[Au(CN)_2]$ with TgSH, PaSH, and TmSH were similarly examined.

^{13}C NMR Spectra of $Au(SGt)_2^{2-}$/KCN Reaction Mixtures. Gold(I) glutathione[20] (94 mg, 187 μmol) and GtSH (61.6 mg, 200 μmol) were dissolved in 3.0 mL of 80% H_2O/20% D_2O to form the water-soluble bis(glutathionato)gold(I) complex. Its spectrum was taken at pH 7. One equivalent of potassium cyanide (13 mg) was added, and a spectrum was obtained. A third spectrum was taken with 2 equiv of KCN in solution. The reaction was repeated with ^{13}C-enriched KCN.

^{13}C NMR Spectra of AuSTm/KCN Reaction Mixtures. The spectrum of AuSTm (0.2 M in 80% H_2O/20% D_2O; pD 7.0) was obtained. One equivalent of potassium cyanide (0.04 g) was added to the mixture, the pD was readjusted to 7.0, and a spectrum was taken. A final spectrum was taken with 2 equiv of KCN. The reaction was repeated with ^{13}C-enriched KCN.

Raman Spectra of $K[Au(CN)_2]$/RSH Reaction Mixtures. Stock solutions of $K[Au(CN)_2]$ (114 mg, 396 μmol) and GtSH (120 mg, 390 μmol) each in 2.0 mL of DD H_2O were prepared. Reaction mixtures prepared with 0.2 M gold(I) and 0, 1, and 2 equiv of GtSH were transferred into a capillary tube and then repetitively scanned from 2030 to 2250 cm^{-1} over 1–2 h. The ν_{CN} band of $Au(CN)_2^-$ at 2172 cm^{-1} and a weaker peak at 2150 cm^{-1} were observed. When a second equivalent of GtSH was added, the intensity of the 2150-cm^{-1} band increased. These measurements were repeated with use of penicillamine and thiomalate.

Raman Spectra of AuSR/KCN Reaction Mixtures. The spectrum of AuSTm (0.2 M in 80% H_2O/20% D_2O; pH 7.0) was obtained. One equivalent of cyanide (6.4 mg of $K^{13}CN$ + 21.1 mg of $K^{12}CN$) was added to the AuSTm solution, the pH was adjusted with $HClO_4$, and another scan was obtained. Another 1 equiv of cyanide was added, and the final spectrum was taken. A mixture of $Au(SGt)_2^{3-}$ and cyanide was examined similarly.

Results

As Brown, Smith, and McKinley[13] observed, the formation of insoluble AuSCy from $Au(CN)_2^-$ was nearly complete (88.4% theoretical yield). The reaction is

$$Au(CN)_2^- + CySH + H^+ \rightarrow (1/n)[AuSCy]_n + 2HCN \quad (3)$$

With *N*-acetyl-L-cysteine, precipitation could be induced only after the aqueous solution was condensed and ethanol was introduced. The yield was only 55% and the elemental analyses were always low for carbon, suggesting some hydrolysis of the acetyl group. In attempts to synthesize the related compounds of glutathione, penicillamine, thiomalate, and thioglucose, no precipitation occurred in aqueous solution and methods similar to that used to isolate (*N*-acetyl-L-cysteinato)gold(I) were unsuccessful. Failure to obtain products would result if the equilibrium of reaction 4 lies far to the left or if the reactants were less soluble than products and precipitated first during evaporation of solvent.

$$Au(CN)_2^- + RSH + H^+ \rightleftharpoons (1/n)[AuSR]_n + 2HCN \quad (4)$$

To determine whether any reaction was occurring in solution (i.e. the position of the equilibrium), ^{13}C NMR spectra were examined. For solutions of $K[Au(CN)_2]$ and GtSH at pH 7, the only peak in the cyanide region was a very weak resonance at 155.8 ppm due to $Au(CN)_2^-$. No signal for cyanide ion (166 ppm) or HCN (117 ppm) was observed. Use of 20 % D_2O/80% H_2O increased intensity of the $Au(CN)_2^-$ resonance, but it was still much less intense than resonances of the glutathione carbons. The "quaternary" carbon of the metal-bound cyanide lacks protons to generate nuclear Overhauser effect (NOE) enhancement and has a T_1 value of 33 s.[23] Free cyanide (HCN at pH 7) or mixed-ligand complexes such as (GtS)$AuCN^-$ would also have long T_1 values. In addition, HCN and $Au(CN)_2^-$ undergo rapid ligand exchange, which may average or broaden their resonances. Thus, the absence of resonances for $GtSAuCN^{2-}$ and HCN was not strong evidence that the equilibrium of reaction 4 lies far to the left.

The resonance of the carbon bound to sulfur in a thiol ligand shifts significantly upon complexation to gold(I).[2,5,17] In the reaction mixture containing equimolar $Au(CN)_2^-$ and GtSH, only a single set of carbon resonances was observed (not shown), indicating either a single form of glutathione in solution or that exchange of free and bound glutathione was rapid on the NMR time scale. Small shifts and pronounced broadening of the glutathione resonances in this solution favor the latter explanation.

In an equimolar mixture of TmSH and $Au(CN)_2^-$ at pH 7 a similar result was obtained: $Au(CN)_2^-$, but no other cyanide species, was detected by natural-abundance ^{13}C NMR. The shifts of the TmSH carbons were reproducibly different from those of free ligand, with only a single resonance for each carbon atom. When penicillamine (PaSH) was used, the C_2 and C_3 methyl resonances shifted reproducibly from 30.6 and 33.2 ppm to 31.1 and 34.3 ppm (not shown). The thiol carbon (C_1) resonance shifted from 46.7 to 47.1 ppm and the α-carbon resonance from 67.5 to 68.1 ppm, and the carboxylate (C_5) resonance was unshifted. The four shifted resonances were broadened, indicative of exchange between a gold-bound penicillamine and free ligand. These spectra were measured in D_2O, and no cyanide resonances were observed.

Direct evidence for an additional species was obtained under acidic conditions. When thiomalic acid (H_2TmSH) was added without neutralization to a similar $Au(CN)_2^-$ solution, new, weaker resonances for C_2 and C_3 were observed on the low-field side of the free ligand resonances, confirming the presence of a new gold(I) thiolate. On the basis of previous findings with AuSTm and the products of its reactions with thiols, we assign the 40.3 ppm resonance of H_2TmSH and 41.0 ppm of the new species, $TmSAuCN^-$, to the C_3 carbon atoms and the corresponding 43.2 and 45.7 ppm resonances to the C_2 carbon atoms.

These results are consistent with the reaction

$$Au(CN)_2^- + RSH \rightleftharpoons RSAuCN^- + HCN \quad (5)$$

with the reactants strongly favored over products. If so, cyanide should displace thiolate ligands from AuSR and $Au(SR)_2^-$. Gold(I) glutathione is not very soluble (200 μM), but a solution containing 0.2 M $Au(SGt)_2^{3-}$ could be formed in situ by reaction of solid AuSGt with an equimolar amount of GtSH. Only a single set of ^{13}C resonances was observed (Figure 3A). They were assigned by analogy to those of free glutathione.[24] The largest shifts were for the cysteinyl resonances C_5 (28.5 to 32.3 ppm) and C_4 (58.4 to 60.4 ppm). The C_5 resonace was broadened, indicating some dissociation of the glutathione according to eq 1. The remaining resonances (29.1, 34.4, 46.3, 57.2, 162.8, 175.0, 176.7, 177.6, 178.9 ppm) were shifted less than 0.5 ppm.

When 1 equiv of cyanide was added to $Au(SGt)_2^{3-}$, the C_5 and C_4 signals shifted toward the values of free GtSH ligand (29.6 and 59.4 ppm). After addition of 2 equiv of CN^-, the resonances were very close to those of free ligand and slightly broadened. Similarly, when an equimolar amount of KCN was added to AuSTm, the 49.1 ppm resonance of C_2 shifted to 45.7 ppm. After a second 1 equiv of CN^- was added, the C_2 resonance was 44.4 ppm, that of free thiomalate. The appearance of a single, shifted

(23) Pesek, J. J.; Mason, R. W. *Inorg. Chem.* **1979**, *18*, 954–957.
(24) Jung, G.; Breitmaier, E.; Voelter, W. *Eur. J. Biochem.* **1972**, *24*, 438–445.

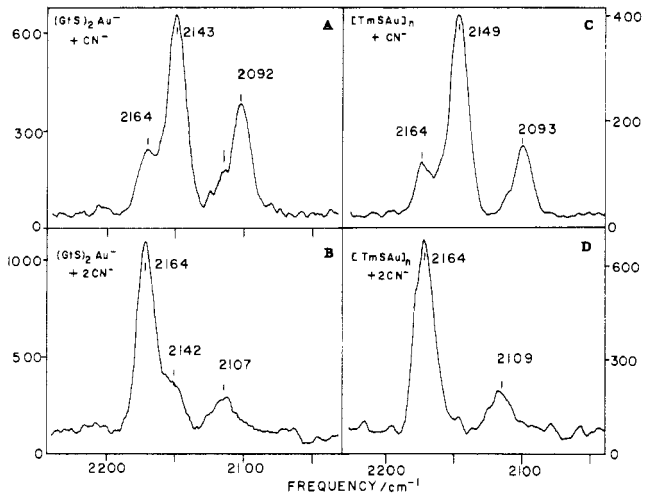


Figure 1. Raman spectra (2040–2230 cm^{-1}) of $RSAuCN^-$ and $Au(CN)_2^-$ in equilibrium: (A) $(GtS)_2Au^{3-}$ + KCN; (B) $(GtS)_2Au^-$ + 2 KCN; (C) $AuSTm^{2-}$ + KCN; (D) $AuSTm^{2-}$ + 2 KCN. Enriched (25%) $K^{13}CN/K^{12}CN$ was used for all spectra.

resonance for each carbon after 1 mol of CN^- is added to either gold complex is consistent with, but not unequivocal evidence for, the formation of the mixed-ligand complex $RSAuCN^-$ and rapid exchange of the bound and free thiol in solution.

Since the rapid ligand exchange complicated the interpretation of the NMR data, Raman spectroscopy was used to investigate the system. Two advantages of this method are the very short time scale (since it is a vibrational technique) and its suitability for studying aqueous systems. The symmetric stretch, ν_{CN}, of aqueous $Au(CN)_2^-$ is an intense Raman band at 2164 cm^{-1} (lit.[25] 2164 cm^{-1}). The asymmetric stretch (2141 cm^{-1}) is symmetry-forbidden in the Raman effect. For HCN, ν_{CN} is at 2080 cm^{-1} and is a much weaker signal. When an equimolar amount of a thiol was added to aqueous 0.2 M $K[Au(CN)_2]$, a new signal appeared between 2140 and 2150 cm^{-1}. In every case, the peak at 2140–2150 cm^{-1} increased in intensity and the $Au(CN)_2^-$ peak decreased after adding a second equivalent of thiol. This result provides strong evidence for the formation of $RSAuCN^-$. The formation of the three-coordinate species $RSAu(CN)_2^{2-}$ can be discounted because the selection rules predict two Raman-active CN bands for a planar molecule. The decreases (ca. 10%) in the intensity of the $Au(CN)_2^-$ band upon successive additions of any thiol confirmed that only a small fraction was converted to $RSAuCN^-$.

To confirm that the lower energy peak was due to a cyanide vibration, Raman spectra were obtained after reaction of 75% $^{12}CN^-$/25% $^{13}CN^-$ with $Au(SGt)_2^{3-}$ (Figure 1A,B). After 1 equivalent of cyanide was added (Au:GtSH:CN^- = 1:2:1), four peaks were observed in the cyanide region: 2164 (sh), 2143 (s), 2107 (sh), and 2092 (m) cm^{-1}. The shoulder corresponds to $Au(^{12}CN)_2^-$, and the major peak at 2149 cm^{-1} is due to $GtSAu^{12}CN^{2-}$. The peaks and shoulder at 2092 and 2107 cm^{-1} are assigned to $GtSAu^{13}CN^{2-}$ and $Au(^{12}CN)(^{13}CN)^-$, respectively. The observed isotopic frequency ratio, 0.9762, for $GtSAuCN^{2-}$ is larger than the predicted ratio, 0.9608. Partial mixing of the Au–C and C–N stretching motions as found in $Au(CN)_2^-$ by Jones[25] can account for the discrepancy. When a second equivalent of cyanide was added, the 2164-cm^{-1} band was predominant and the 2143-cm^{-1} band was a shoulder (Figure 1B). The band at 2092 cm^{-1} was very weak, and the peak due to $Au(^{12}CN)(^{13}CN)^-$ at 2107 cm^{-1} had gained intensity. Thus, at a Au:GtSH:CN^- ratio of 1:2:2, the major gold species was $Au(CN)_2^-$ and some $GtSAuCN^{2-}$ was also present.

Similar results were obtained with AuSTm and CN^-, after allowance for the different stoichiometric ratios of reactants: the Au:TmSH:CN^- ratio was 1:1:1 in the initial reaction (Figure 1C)

(25) Jones, L. H. *J. Chem. Phys.* **1957**, *27*, 468–472.

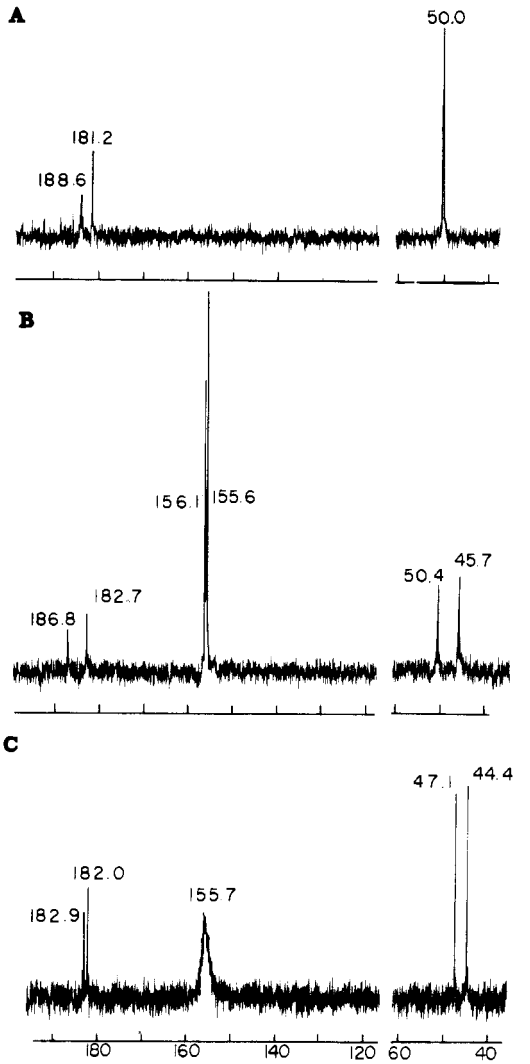


Figure 2. ^{13}C NMR spectra of thiomalate/gold/cyanide (25% ^{13}C enriched) equilibrium mixtures: (A) $AuSTm^{2-}$; (B) $AuSTm^{2-}$/KCN; (C) $AuSTm^{3-}$/2 KCN.

and 1:1:2 in the second reaction (Figure 1D). The major bands after addition of 1 equiv of CN^- were at 2149 and 2093 cm^{-1} for the ^{12}C and ^{13}C isotopic species of $TmSAuCN^{3-}$. The reduced thiol content led to complete loss of the $TmSAuCN^{3-}$ bands when 2 equiv of cyanide was added, indicating essentially complete conversion to $Au(CN)_2^-$.

To verify the finding of the Raman studies, that $RSAuCN^-$ is present, the NMR studies were repeated by use of $K^{13}CN$ with $AuSGt^-$, $Au(SGt)_2^{3-}$, and $AuSTm^{2-}$. As shown in Figure 2, at a 1:1:1 ratio of Au:TmSH:CN^-, two strong resonances appeared at 156.1 and 155.7 ppm. They are assigned to $Au(CN)_2^-$ and $TmSAuCN^{3-}$. The presence of $Au(CN)_2^-$ indicated that significant disproportionation of $TmSAuCN^{3-}$ was occurring. After the second equivalent of CN^- was added, only a single very broad cyanide peak, 155.7 ppm, was observed, indicating cyanide equilibration between several distinct environments. The resonances of the thiomalate carbons were identical with those observed for $K^{12}CN$ and described above. At this stoichiometry, Au:TmSH:CN^- = 1:1:2, $Au(CN)_2^-$ dominates the equilibrium, with traces of $TmSAuCN^{3-}$ and HCN formed by competition of the thiomalate for the gold causing exchange broadening of the cyanide resonance.

It was found that $AuSGt^-$ would dissolve in the presence of equimolar KCN after adjusting the pH to 7. The spectrum contained two sharp cyanide resonances at 155.8 and 154.8 ppm, which result from an equilibrium mixture of $Au(CN)_2^-$ and $GtSAuCN^{2-}$. Single resonances for the cysteinyl carbons of glutathione, C_5 and C_4 at 31.4 and 60.4 ppm, arise from rapid equilibration of $GtSAuCN^{2-}$ and $Au(SGt)_2^{3-}$. Thus, as for the

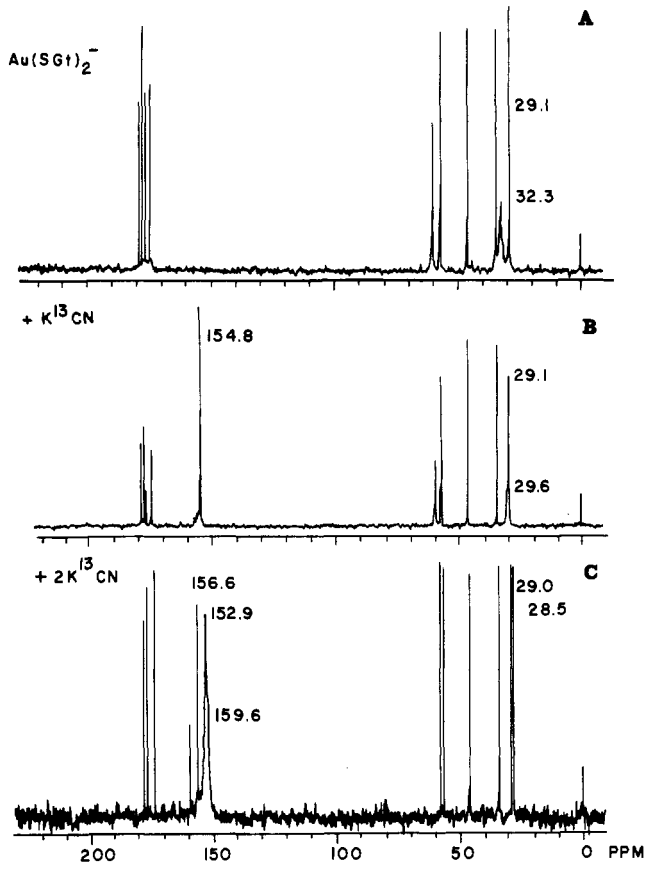


Figure 3. ^{13}C NMR spectra of glutathione/cyanide/gold equilibrium mixtures: (A) $Au(SGt)_2^{3-}$; (B) $Au(SGt)_2^{3-}$/KCN; (C) $Au(SGt)_2^{3-}$/2KCN. The peaks at 156.6 and 159.6 ppm in (C) arise from direct reaction between glutathione disulfide (an impurity in GtSH) and cyanide (see text).

thiomalate system, the dominant species at the 1:1:1 $Au:CN^-$:GtSH ratio is $GtSAuCN^{2-}$, which partially disproportionates to $Au(CN)_2^-$ and $(GtS)_2Au^{3-}$. The reactions of $AuSTm^{3-}$ and $AuSGT^-$ with KCN are

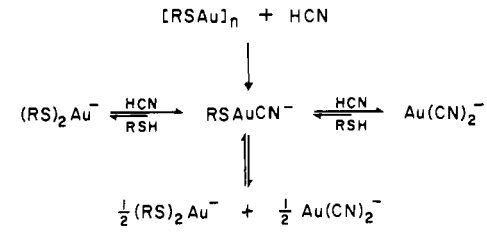
$$AuSR + CN^- \rightarrow RSAuCN^- \rightarrow (RS)_2Au^- + Au(CN)_2^-$$

When equimolar $K^{13}CN$ was added to $Au(SGt)_2^{3-}$ formed in situ (Figure 3B), a single sharp band, 154.8 ppm, was present in the cyanide region. Since the Raman spectrum of an identical mixture (Figure 1A) demonstrates that $Au(CN)_2^-$ and $GtSAuCN^{2-}$ are the minor and major products, this resonance must be the averaged signal of these two species. When a second equivalent of $K^{13}CN$ was added (Figure 3C), the gold-bound cyanides appeared as a single broad resonance centered at 152.9 ppm. The shift (2.9 ppm) indicates that a substantial concentration of HCN has formed and is in rapid exchange with $Au(CN)_2^-$ and $GtSAuCN^{2-}$. With use of $\delta_C = 155.8$ for $Au(CN_2)_2^-$, 117.0 ppm for HCN, and 154.8 ppm for GtSAuCN, an estimate of 0.08 is obtained for the mole fraction of gold present as the mixed-ligand complex.

The resonances at 156.6 and 159.6 ppm can be generated by direct reaction of $K^{13}CN$ and $GtSSGt^{2-}$, an impurity (4%) in glutathione. These minor products appear intense because they are 99% ^{13}CN enriched. The thiazole previously postulated as a product of this reaction[26] does not explain the ^{13}C-labeled sp^2 carbon atoms (δ = 156.6 and 159.6) nor the deuterium incorporation, J_{DC} = 31 Hz, observed here. This reaction is under further investigation.

Using Chernyak and Shestopalova's[27] electrode potential, $E^\circ_{1/2}$ = 0.144 V, for the Au(0)/Au(I) couple in the presence of cysteinate ion (CyS^-) and the standard Au(0)/Au(I) potential and correcting for the pK_a of cysteine, one can calculate a value for K_7:

Scheme I



$$2CyS^- + Au^0 \overset{E^\circ_1}{\rightleftharpoons} Au(SCy)_2^- + e^- \quad (6)$$

$$Au^0 \overset{E^\circ_2}{\rightleftharpoons} Au^+ + e^-$$

$$CySH \overset{K_a}{\rightleftharpoons} CyS^- + H^+$$

$$2CySH + Au^+ \overset{K_7}{\rightleftharpoons} Au(SCy)_2^- + 2H^+ \quad (7)$$

$$K_7 = (K_a)^2 e^{nF(E^\circ_1 - E^\circ_2)/RT}$$
$$= 3 \times 10^{14}$$

At pH 7 the conditional equilibrium constant, K_7^{pH7}, is 3×10^{28}, indicating a very favorable reaction. Furthermore, these data can be used with the value of K_1 to calculate the equilibrium constant $K_2 = K_7/K_1 = 1.5 \times 10^{17}$. The conditional equilibrium constant at pH 7 is then found to be $K_1^{pH7} = 1.5 \times 10^{24}$.

Discussion

The results described here indicate that the reactions of cyanide with mono- and bis(thiolato)gold(I) species generate detectable concentrations of $RSAuCN^-$. To our knowledge, there is only one previous report of a similar species: $O_3S_2AuCN^{2-}$ (ν_{CN} = 2140 cm^{-1}), formed by dissolving AuCN in thiosulfate solution.[28] The amount of gold present as $RSAuCN^-$ will depend strongly on the proportions of gold, thiolate, and cyanide in solution. The equilibria involved are described in Scheme I. All the species except $[AuSR]_n$ are present in the equilibria established with $Au:CN^-$:RSH ratios between 1:1:2 and 1:2:1. It is expected that the relative affinities of various thiols for gold will shift the positions of the equilibria, but more subtly than the qualitative changes found here.

At pH 7 the exchange of free and bound thiol was rapid on the ^{13}C NMR time scale with penicillamine, glutathione, and thiomalate. The rapid exchange between $TmSAuCN^{3-}$ and $TmSH^{2-}$ contrasts with the slow exchange previously observed between $(TmS)_2Au^{5-}$ and $TmSH^{2-}$.[2,19,29] This result suggests that the charge of the three-coordinate transition states for exchange (−6 and −8, respectively) as well as the previously identified factors of solution pH and thiol pK_a[5] are important in determining the rate of exchange. $Au(CN)_2^-$ and $TmSAuCN^{3-}$ were well-resolved, indicating that exchange between the two gold-bound forms of cyanide was slow. When excess HCN was present, however, rapid exchange of free and bound cyanide was observed.

The reaction of cysteine with $Au(CN)_2^-$ to form AuSCy is unique among the thiols examined. It is driven by the insolubility of AuSCy, which at neutral pH has a solubility of only 1–2 μM.[4] For other thiols, including acetylthioglucose (ATgSH), which forms a water-insoluble complex (AuSATg), no spontaneous precipitation of the corresponding AuSR species occurs. Even by concentration of the aqueous reaction mixtures and addition of less polar solvents, a product was isolated only in the case of *N*-acetylcysteine. Formation of (cysteinato)gold (I), but not other thiol analogues, has been observed in other reactions of gold(I)

(26) Catsimpoolas, N.; Wood, J. L. *J. Biol. Chem.* **1966**, *241*, 1790–1796.
(27) Chernyak, A. S.; Shestopalova, L. F. *Russ. J. Inorg. Chem.* (*Engl. Transl.*) **1976**, *21*, 464–465.
(28) Mayer, B.; Hinnawi, M. *Abstr. Pap.—Am. Chem. Soc.* **1979**, *178th*, INOR 90.
(29) Shaw, C. F., III. In "Bioinorganic Chemistry of Gold Coordination Compounds"; Sutton, B. M., Ed., Smith Kline and French Laboratories: Philadelphia, 1983; pp 98–123.

compounds: reaction of cystine and AuSTm,[30] reaction of cysteine and AuSTm,[18] and spontaneous decomposition of $(C_6H_5)_3PAuSCy$.[31] The factors causing the very low solubility of AuSCy at neutral pH are unknown, but they apparently drive many reactions involving gold and cysteine to completion.

Given the large values for the equilibrium formation constant of $Au(CN)_2^-$ in the literature (4×10^{28} or 10^{39}),[20,21] the presence of substantial amounts of $RSAuCN^-$ at equilibrium in the presence of 2 mol of cyanide was somewhat unexpected. The estimated formation constants for AuSCy and $Au(SCy)_2^-$, however, demonstrate substantial thermodynamic stability for these species. Stability constants for other gold(I) thiolates are presumably of similar magnitude, and hence, formation of $RSAuCN^-$ by thiol displacement of CN^- is thermodynamically feasible, but not favorable.

Graham et al.[9] examined the reactions of gold thioglucose and $AuSTm^{2-}$ with cyanide using UV spectral changes and chemical analysis of HCN and concluded that the reaction is dominated by formation of $Au(CN)_2^-$. While this conclusion holds at CN^-/Au ratios approaching or exceeding 2.0, the probes used here, which are more sensitive to the molecular species involved, demonstrate that the mixed-ligand complexes such as $TmSAuCN^{3-}$ are important at lower CN^-/Au ratios. The formation of $RSAuCN^-$ in equilibrium with $Au(CN)_2^-$ explains their observation of volatile cyanide, HCN, before the CN^-/Au ratio reaches 2.0 (Figure 6 of ref 9).

In the blood, the concentration of protein-bound gold species such as the serum albumin complex AlbSAuSR (RSH = GtSH, CySH, TmSH, etc.) is likely to exceed the cyanide concentration, so that $CN^-/Au \leq 1.0$. Under these conditions AlBSAuCN and $RSAuCN^-$, etc., may be the major species present and contribute to the transport of gold across cell membranes into red blood cells.

Acknowledgment. We wish to thank Frank Laib and Dr. Suzanne Wehrli for their assistance in obtaining the NMR spectra and Smith Kline and French Laboratories for financial support. We thank Professor G. C. Stocco for helpful discussions under the auspices of NATO Grant No. 890/83.

Registry No. $Au(CN)_2^-$, 14950-87-9; CN^-, 57-12-5.

(30) Danpure, C. J.; Lawson, K. J. *Biochem. Soc. Trans.* **1977**, *5*, 1366-1368.
(31) Coates, G. E. Kowala, C.; Swan, J. M. *Aust. J. Chem.* **1966**, *19*, 539-545.

Contribution from the Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan

^1H NMR Study of Aromatic Ring Stacking in Ternary Palladium(II) Complexes Involving Aromatic Diamines and Dipeptides with N-Terminal Aromatic Amino Acid Residues

Akira Odani, Shuhei Deguchi, and Osamu Yamauchi*

Received August 8, 1985

A ^1H NMR spectroscopic study on intramolecular aromatic ring stacking has been made for the ternary palladium(II) complexes Pd(L)(DA), where L refers to dipeptides with the N-terminal aromatic amino acids tyrosylglutamate, tyrosylglycinate (Tyr-Gly), tryptophylglutamate (Trp-Glu), tryptophylglycinate (Trp-Gly), phenylalanylglutamate, or phenylalanylglycinate and DA refers to 2,2′-bipyridine (bpy), 4,7-diphenyl-1,10-phenanthroline-4′,4″-disulfonate (bphen), or ethylenediamine (en). The ternary complexes have a planar N_4 coordination structure, and the fractional populations of the three staggered rotamers calculated from the coupling constants indicated that the rotamer capable of intramolecular ring stacking is favored in Pd(L)(DA) (DA = bpy or bphen) as compared with Pd(L)(en), which does not involve the stacking. Upfield shifts $\Delta\delta$ of the ring proton signals of L due to proximal aromatic rings of DA (0.66–1.27 ppm for H-3 of the tyrosine phenol ring) have substantiated the stacking interaction. From the observed $\Delta\delta$ values and the values for complete stacking, the stability, log K, of the stacking in Pd(L)(DA), which is related to the constant K_{ST} for the "unstacked form $\rightleftharpoons$ stacked form" equilibrium by $K = K_{ST} + 1$, has been evaluated to be high for L = Trp-Gly (1.73), Trp-Glu (0.95), and Tyr-Gly (0.95) at 25 °C, the orders being indole > phenol > benzene > phenolate as expressed by the side chain aromatic ring of L and bphen > bpy. Temperature dependences of log K values have revealed that the enthalpy change contributes to the stacking interaction. Dissociation of the tyrosine phenol OH group of L weakens the aromatic ring stacking, with a log K decrease of 0.26–0.73, indicating that the stacking is regulated by introducing a charged substituent.

Introduction

Ligand–ligand interactions in mixed-ligand–metal complexes as a source of ligand discrimination that leads to specificity or selectivity of ternary complex formation have attracted much attention in recent years for their relevance to biological reactions involving metal ions at the active sites.[1] Ligand specificity arising from the interactions in ternary complexes may be regarded as a prototype of the substrate specificity of metalloenzymes and may offer a key to elucidating metal ion mediated specific interactions between biomolecules. It may also give information on the processes of biological recognition both in the absence and the presence of metal ions,[2] because metal ions serve as a probe giving various signals, and the coordination structure may mimic the stereochemistry at the recognition site.

We have been interested in the copper(II) complex formation by tyrosine-containing peptides[3] as models for endogenous peptides with morphine-like analgesic activity (opioid peptides) such as enkephalin and endorphin, all of which have the essential N-terminal tyrosyl residue with a phenol group capable of binding with copper(II)[3,4-9] and stacking with aromatic rings.[10-12] The

(1) (a) Sigel, H. *Met. Ions Biol. Syst.* **1973**, *2*, 63-125. (b) Sigel, H. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 394-402. (c) Pettit, L. D.; Hefford, R. J. W. *Met. Ions Biol. Syst.* **1979**, *9*, 173-212.
(2) Frieden, E. *J. Chem. Educ.* **1975**, *52*, 754-761. (b) Jencks, W. P. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1975**, *43*, 219-410.
(3) Yamauchi, O.; Tsujide, K.; Odani, A. *J. Am. Chem. Soc.* **1985**, *107*, 659-666.
(4) Hefford, R. J. W.; Pettit, L. D. *J. Chem. Soc., Dalton Trans.* **1981**, 1331-1335.
(5) Bogess, R. K.; Martin, R. B. *J. Am. Chem. Soc.* **1975**, *97*, 3076-3081.
(6) Pastor, J. M.; Garnier, A.; Tosi, L. *Inorg. Chim. Acta* **1979**, *37*, L549-L550.
(7) Fazakerley, G. V.; Linder, P. W.; Torrington, R. G.; Wright, M. R. W. *J. Chem. Soc., Dalton Trans.* **1979**, 1872-1880.
(8) Garnier-Suillerot, A.; Albertini, J.-P.; Collet, A.; Faury, L.; Pastor, J.-M.; Tosi, L. *J. Chem. Soc., Dalton Trans.* **1981**, 2544-2549.
(9) Kiss, T.; Gergely, A. *J. Chem. Soc., Dalton Trans.* **1984**, 1951-1957.
(10) Fischer, B. E.; Sigel, H. *J. Am. Chem. Soc.* **1980**, *102*, 2998-3008.
(11) Yamauchi, O.; Odani, A. *Inorg. Chim. Acta* **1985**, *100*, 165-172.

0020-1669/86/1325-0062$01.50/0