Notes

Contribution from the Departments of Chemistry, San Jose State University, San Jose, California 95192, and Northern Illinois University, DeKalb, Illinois 60115

Detection of Mercury Coordination Numbers Greater Than 2 for Organomercurials Using Chlorine-35 Magnetic Resonance

Joseph J. Pesek*† and John F. Schneider‡

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Of all the organomercurial compounds, the coordination chemistry of CH_3Hg^{II} has probably been the most extensively studied.[1] In most cases the solution chemistry of methylmercury, and other organomercurials, can be described by linear 2-coordination of the mercury. However, there are a few examples of higher mercury coordination numbers. Most notable are the $CH_3HgX_2^-$ and $CH_3HgX_3^{2-}$ complexes of iodide[2] and thiocyanate.[2-4] A few chelating ligands[5-9] such as 2,2′-bipyridine have been shown to have mercury coordination numbers greater than 2. There are no reports in the literature showing that methylmercury displays any higher coordination with chloride in aqueous solution. One previous study involving higher coordination iodide and thiocyanate complexes[2] found no evidence for similar methylmercury complexes with chloride or bromide. However, spectroscopic studies have shown that $CH_3HgCl_2^-$ is formed in ethanol[10] as well as benzene, chloroform, dichloromethane, tetrahydrofuran, and acetonitrile.[11]

One interesting possibility for the detection of such complexes, if they exist, involves the use of the halide ion NMR technique for monitoring mercury binding to a protein.[12-15] In this method broadening of the halide magnetic resonance line occurs due to a large distortion of the electric field gradient at the nucleus producing efficient quadrupolar relaxation. These effects are magnified by the relatively slow reorientation of the field when the halide is bound to a mercury–protein complex. When exchange between the bulk chloride (line width ~ 10 Hz) and the bound chloride (line width $\sim 10^6$ Hz) occurs at the appropriate rate, it produces measurable line broadening because the resonance observed is a weighted average of the two environments. This has been referred to as a "chemical amplification effect".[16] The experimental conditions involve halide ion concentrations in the range 0.1–1.0 M and mercury–protein concentrations of about 10^{-5} M. Therefore, the halide:metal ratio is at least 10^4, which would favor detection of weak higher coordination number complexes assuming that the overall halide ion exchange rate does not fall outside the proper kinetic window for line broadening to occur. In addition, the larger mass of the organomercurial as well as changes in the halide ion exchange rate between the metal bound and bulk chloride can lead to increased line broadening,[17] which would improve the sensitivity for detecting weak complexes. Therefore, with the use of methylmercury or another organomercurial instead of Hg^{2+} to complex a protein in the presence of halide ion, the formation of a protein–organomercurial–halide species can be detected by an increase in the halide ion NMR line width.

Figure 1 is a plot of the chlorine-35 line width at 9.75 MHz for a 0.1 M NaCl solution in the presence of 10^{-5} M insulin at pH 8.0 and various mercury species. The experimental procedures used have been previously described.[15] As expected, Hg^{2+} produces substantial line broadening upon formation of the mercury–insulin complex, causing exchange of the halide between free chloride

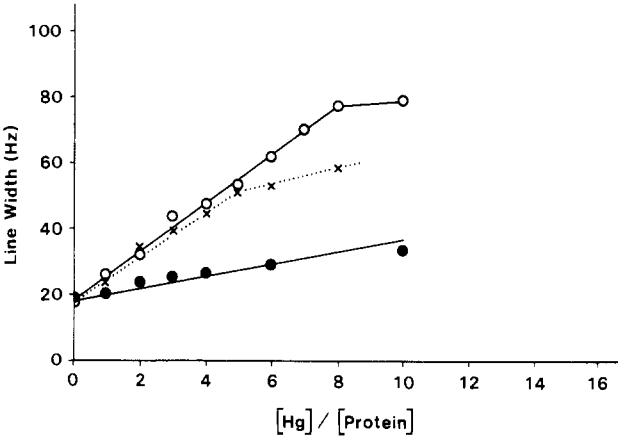

Figure 1. Chlorine-35 line width (Hz) vs. mercury:protein ratio for a 0.1 M NaCl solution in the presence of 10^{-5} M insulin with the addition of mercury (○), methylmercury (●), and PMB (×).

in solution and bound chloride in the metal–protein complex. These results indicate several mercury-binding sites (possibly as many as eight) on insulin.

The increase for methylmercury, while not as great as that of mercury, is substantial. The more than 50% increase in line width (from 18 to 31 Hz) when 10 equiv of CH_3Hg^+ is added is significant since no increase is observed in an identical chloride solution in the absence of protein. Furthermore, fluorescence studies[15] support binding of methylmercury to insulin and the other proteins in this study and the theory of the halide probe method[18] precludes significant line broadening without strong attachment, i.e. covalent bonding, between the metal and the protein. The only other possibility for line broadening, which has been established experimentally, is a substrate that is mercury labeled.[19] Because one coordination site is occupied by the methyl moiety and a second site by the protein donor atom, a third site is required for chloride to exchange between the metal and the bulk of solution. The smaller increase in line width for methylmercury in comparison to mercury is probably partly due to the lower formation constant for chloride addition to the CH_3Hg^+–protein complex (K_3) than to the Hg^{2+}–protein complex (K_2).

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†San Jose State University.
‡Northern Illinois University.

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Table I. Chlorine-35 Line Broadening for Mercury Species in the Presence of Various Proteins

protein	line width increase,[a] Hz		
	Hg^{2+}	CH_3Hg^+	PMB
insulin[b]	42 (8:1)[d]	10 (ND)[e]	32 (6:1)[d]
papain[c]	47 (4:1)[d]	5 (ND)	36 (6:1)[d]
α-chymotrypsin[c]	21 (6:1)[d]	9 (ND)	41 (ND)
alkaline phosphatase[b]	59 (5:1)[d]	6 (ND)	27 (ND)

[a] 6:1 metal:protein ratio. [b] pH 8.0. [c] pH 5.0. [d] Apparent stoichiometry of Hg–protein complex. [e] ND = not possible to determine stoichiometry of complex.

Also shown on Figure 1 is the curve for *p*-(chloromercuri)-benzoic acid (PMB). This organomercurial exhibits significantly greater line broadening than methylmercury. However, in PMB, as in CH_3Hg^+, one mercury coordination site is occupied by the protein and the second is occupied by the organic moiety. Therefore, for line broadening to occur, chloride must occupy a third coordination site. The greater line broadening for PMB vs. CH_3Hg^+ could be due to one or more of the following: (1) stronger binding to the protein, (2) stronger association of chloride ion, (3) a faster chloride-exchange rate, or (4) a longer correlation time. It is certain that (4) must be a factor due to the larger mass of organic moiety as well as the fact that the ionized carboxylate group could be weakly attracted to other parts of the protein. The 6:1 stoichiometry seen for PMB may be due to weaker binding of PMB to insulin than to Hg^+ or perhaps to the steric obstruction of the benzoic acid moiety, which might cause a significantly slower halide exchange at two binding sites.

Table I is a summary of the observed chlorine-35 line broadening for mercury, methylmercury, and PMB in the presence of several proteins. In all cases, binding of the organomercurial to the protein is confirmed by fluorescence measurements. For all proteins with equivalent amounts of organomercurial, line broadening is greater for PMB than for methylmercury. Again it seems probable that (4) is at least partly responsible for this effect. Reinforcing the assumption are the results for α-chymotrypsin with PMB, producing significantly greater line broadening than Hg^{2+}. Additional studies are under way to determine if similar bromide complexes exist as well as to measure the correlation times and the halide ion exchange rates for these species.

Registry No. Hg^{2+}, 14302-87-5; CH_3Hg^+, 22967-92-6; PMB, 59-85-8; insulin, 9004-10-8; papain, 9001-73-4; chymotrypsin, 9004-07-3; alkaline phosphatase, 9001-78-9.

Contribution from the Department of Chemistry,
Université du Québec à Montréal,
Montréal, Canada H3C 3P8

Synthesis of Monoamine Platinum(II) Complexes and Crystal Structure of Potassium Trichloro(isopropylamine)platinate(II) Hemihydrate

F. D. Rochon,* R. Melanson, and Monique Doyon

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Although $K[Pt(NH_3)Cl_3]$[1,2] and $K[Pt(py)Cl_3]$[3,4] (py = pyridine derivative) have been known for many years, the methods used to synthesize these complexes are not suitable for monoamine platinum(II) compounds. Kukushkin et al.[5,6] prepared some complexes $[Pt(NH_3)_4][Pt(am)Cl_3]_2$ (am = amine) from $[Pt(Me_2SO)(am)Cl_2]$ through a very long procedure of oxidation, replacement, and reduction. A method for the preparation of mononucleoside platinum complexes by the direct reaction of K_2PtCl_4 and the nucleoside in DMF has been reported,[7] but the method is not applicable to amine complexes. We have recently reported the synthesis of such compounds from the reaction of K_2PtCl_4 with the amine in aqueous solution in the presence of KCl.[8] But the method is strictly limited to bulky amines like *tert*-butylamine and isopropylamine because of the rapid formation of the disubstituted compound $[P(am)_2Cl_2]$.

Monoamine platinum(II) compounds are important since they can be the starting material for the synthesis of the mixed-ligands complexes $[Pt(am)(L)Cl_2]$. If the reaction is done in water and the conditions strictly controlled, only the cis isomer will be formed since the trans effect follows the order Cl > amine.

$$[Pt(am)Cl_3]^- + L \xrightarrow{H_2O} cis\text{-}[Pt(am)(L)Cl_2] + Cl^-$$

Cis mixed-ligand complexes are important especially in view of the potent antitumor activity of cis Pt(II) complexes. If compounds with two different neutral ligands could be systematically synthesized, the screening range of platinum complexes would be largely extended and antitumor activity, toxicity, and solubility could be significantly modified.

We have now developed a new method for the synthesis of ionic complexes $[Pt(am)Cl_3]^-$ that should be applicable to almost all amines or other nitrogen ligands. This method is described below. The compound with isopropylamine gave crystals suitable for X-ray diffraction. Its crystalline structure was studied, and the results are now reported.

Experimental Section

K_2PtCl_4 was bought from Johnson Matthey and Co., Limited, and was recrystallized in water before use. The elemental analyses were performed by Galbraith Laboratories. The IR spectra were measured on a Perkin-Elmer 783 or Digilab FT50 instrument (CsI beam splitter). The ^1H NMR spectra were recorded on a Varian EM-360L instrument (concentration about 0.05 M).

$[Pt(am)I_2]_2$. These compounds were synthesized as already reported.[9]

Synthesis of $K[Pt(am)Cl_3]$. One millimole of the iodo-bridged dimer $[Pt(am)I_2]_2$ was suspended in ~15 mL of water to which 6 mmol of $AgNO_3$ was added. The mixture was stirred at room temperature in the dark for 2 days. The precipitate (AgI) was filtered out, and 8 mmol of KCl were added to the filtrate. The mixture was stirred for 24 h, and the precipitate (AgCl) was removed by filtration. The filtrate was evaporated to dryness and the residue was dried under vacuum for 24 h. The yellow product was dissolved in acetone, and the precipitate (KCl and KNO_3) was filtered out. The filtrate was evaporated to dryness and the residue dissolved in water. The mixture was again filtered and the filtrate evaporated to dryness. The residue was dried under vacuum.

$K[Pt(CH_3NH_2)Cl_3]\cdot{}^1/_2H_2O$: Yield 80%; dec pt 164–177 °C. Anal. Calcd: C, 3.15; H, 1.58; N, 3.67; Cl, 27.97. Found: C, 3.59; H, 1.39; N, 4.00; Cl, 27.99.

$K[Pt(C_2H_5NH_2)Cl_3]\cdot{}^1/_2H_2O$: Yield 80%; dec pt 170–183 °C. Anal. Calcd C, 6.08; H, 2.04; N, 3.55; Cl, 26.94. Found: C, 6.56; H, 2.06; N, 3.86; Cl, 26.72.

$K[Pt(i\text{-}C_3H_7NH_2)Cl_3]\cdot{}^1/_2H_2O$: Yield 65%; dec pt 185–209 °C. Anal. Calcd C, 8.81; H, 2.47; N, 3.43; Cl, 26.03. Found: C, 9.03; H, 2.64; N, 3.32; Cl, 25.91.

$K[Pt(c\text{-}C_4H_7NH_2)Cl_3]\cdot{}^1/_2H_2O$: Yield 45%; dec pt 158–198 °C. Anal. Calcd C, 11.46; H, 2.63; N, 3.32; Cl, 25.27. Found: C, 11.46; H, 2.77; N, 3.44; Cl, 24.32.

$K[Pt(c\text{-}C_5H_9NH_2)Cl_3]\cdot{}^1/_2H_2O$: Yield 65%, dec pt 172–190 °C. Anal. Calcd C, 13.81; H, 3.01; N, 3.22; Cl, 24.46. Found: C, 14.25; H, 3.15; N, 3.49; Cl, 23.67.

$K[Pt(NH_3)Cl_3]\cdot H_2O$: Yield 50%. X-ray diffraction methods have shown that this compound was identical with the one reported earlier.[2]

Crystal Data: $[Pt(i\text{-}C_3H_7NH_2)Cl_3]\cdot{}^1/_2H_2O$, fw = 408.67, monoclinic, $C2/c$, a = 29.153 (24) Å, b = 5.928 (3) Å, c = 12.178 (7) Å, β = 96.95 (6)°, V = 2089 (2) Å^3, $F(000)$ = 1496, D_c = 2.598 Mg m^{-3}, Z = 8, λ(Mo Kα) = 0.71069 Å, μ(Mo Kα) = 146.9 cm^{-1}, and T = 293 K.

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