For a real $Mo_{10}O_{32}$ chain, these π bands occur in pairs since the pair of subchains Ia and IIa interact weakly with that of subschains Ib and IIb. When normalized to $A_3Mo_{10}O_{30}$ (half the unit cell of the blue bronze), the bottom two d-block bands become roughly three-fourths filled. This simple picture of the electronic structure of the blue bronze is modified somewhat for a real $Mo_{10}O_{30}$ slab, because the interaction between subchains Ia and Ib via the Mo(Ia)–O–Mo(Ib) linkage of two adjacent $Mo_{10}O_{32}$ chains is stronger than that between subchains Ia and IIa (or Ib and IIb) within an $Mo_{10}O_{32}$ chain. Thus for an $Mo_{10}O_{30}$ slab, the first and fourth d-block bands (from the bottom) have stronger outer subchain (Ia, Ib) character whereas the second and third bands have stronger inner subchain (IIa, IIb) character. Nevertheless, the bottom two d-block bands of a real $Mo_{10}O_{30}$ slab become partially filled with three electrons for the d-block bands per $A_3Mo_{10}O_{30}$. The upper and lower Fermi surfaces of the first band are found to be nested to the lower and upper Fermi surfaces of the second band, respectively, by the identical wave vector $q_b = 0.75b^*$. This explains why there occurs a single CDW in the blue bronze, as proposed by Pouget et al.[21] For a real $Mo_{10}O_{30}$ slab, the third d-block band lies only 0.012 eV above the Fermi level. Thus, population of the bottom of the third band by thermal excitation from the bottom two bands will decrease the q_b value of the latter bands. As shown by Pouget et al.,[21] this accounts for the temperature dependence of q_b in the blue bronze, which increases gradually from $\sim 0.72b^*$ at room temperature to $\sim 0.75b^*$ below T_c.

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Variable-Temperature ^{195}Pt NMR Spectroscopy, a New Technique for the Study of Stereodynamics. Sulfur Inversion in a Platinum(II) Complex with Methionine

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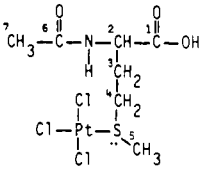
This paper introduces ^{195}Pt NMR spectroscopy as a method for the study of stereodynamics and apparently represents only the third report on variable-temperature ^{195}Pt NMR spectroscopy. Displacement of a Cl^- ligand from $PtCl_4^{2-}$ by *N*-acetyl-L-methionine (AcMetH) yields $[Pt(AcMetH)Cl_3]^-$ (**1**), which is a model for the binding of $PtCl_3^-$ label to proteins. The complex in solution is characterized by the method of preparation and by its UV–vis and ^1H, ^{13}C, and ^{195}Pt NMR spectra. The AcMetH ligand is coordinated to the Pt(II) atom through the S atom in the side chain; coordination of the amide N atom, which would result in a six-membered ring, does not occur. We conclude that the coordination of the amide nitrogen to platinum is facilitated if it yields a five-membered ring or a larger chelate containing such a ring. On account of its chiral S atom, complex **1** exists in two diastereomeric forms, which are undetectable in the ^1H and ^{13}C NMR spectra at convenient temperatures but are clearly evident in the ^{195}Pt NMR spectrum. The ^{195}Pt NMR spectra at nine temperatures spanning 86 deg show reversible, intramolecular inversion of configuration at the S atom; $\Delta G^* = 63.7$ kJ mol^{-1} at 335 K. Other mechanisms for the interconversion of the diastereomers are ruled out. Since the chiral carbon and sulfur atoms are three bonds apart, there is virtually no stereochemical discrimination and the two diastereomers of **1** exist in equal concentrations. This finding is discussed in terms of the known molecular structures of methionine and its derivatives. We point out that ^{195}Pt NMR spectroscopy is uniquely suited to the study of dynamic processes involving relatively complex biomolecules and processes causing subtle changes in molecular structure.

Introduction

Selective binding of metal complexes to proteins and other biological macromolecules opens various possibilities for the study of these macromolecules. Suitable metals that are covalently attached to protein surfaces can serve as spectroscopic probes of structure and dynamics, redox probes of electron-transfer reactivity, anomalous scatterers for X-ray crystallography, and modifiers of drug action. We have shown[1] that the chloro(2,2′:6′,2″-terpyridine)platinum(II) complex, $[Pt(trpy)Cl]^+$, possesses the required stability, reactivity, and spectroscopic properties to be a useful labeling reagent for selected histidine, cysteine, and arginine residues in cytochromes *c* from horse, tuna, and baker's yeast. These studies in our laboratory were prompted by an earlier discovery that the $PtCl_4^{2-}$ complex binds covalently to exposed methionine residues in proteins[2] and by the subsequent widespread use of this complex as a heavy-atom label for X-ray determination of protein structure.[3,4] Our ultimate goal is to apply ^{195}Pt NMR spectroscopy to platinum-labeled proteins and study the motions of the flexible side chains of amino acid residues at the protein surface. Since the ^{195}Pt NMR signal of a platinum atom bonded to a biological macromolecule has never been observed,[4,5] we first studied the complex $[Pt(AcMetH)Cl_3]^-$ (**1**; AcMetH = *N*-acetyl-L-methionine). This complex is a model



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for the attachment of the $PtCl_3^-$ label to the side chain of the methionine residue in proteins. Relatively few amino acid complexes of platinum are known,[6–9] and like those of other metals,

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most of them contain amino acids as chelating ligands. In particular, there are only three other examples of methionine coordination to platinum solely through the sulfur atom, and they have been incompletely characterized.[5,8,9]

Inversion of pyramidal configuration at coordinated chalcogen atoms (S, Se, and Te) has been observed in various metal complexes.[10] Virtually all of these studies are based on the principle that inversion causes an interchange of diastereotopic H atoms in the prochiral CH_2 group and consequently gives rise to the collapse of an AB quartet into a singlet in the ^1H NMR spectrum. Reliance on this stereochemical principle, and on the corresponding ^1H NMR technique, has in effect restricted previous studies to complexes containing relatively simple ligands of the $(RCH_2)_2S$ and RCH_2–S–CH_2R' types and to the corresponding bidentate ligands, for which the collapse of the methylene quartet upon heating is easily discernible. Species **1** is already too complex to be studied by the established methods, and a platinum-labeled protein would be wholly beyond the applicability of any technique that depends on the fine changes in the pattern of proton signals. Attempts to merely observe changes in the static ^1H NMR spectrum of a protein upon its labeling with the $PtCl_3^-$ complex have shown how unreliable this technique can be even when the protein in question is small, its structure known in detail, and its ^1H NMR spectrum relatively well understood.[11,12]

In this report we show that ^1H and ^{13}C NMR spectroscopy cannot be used to monitor the sulfur inversion in complex **1** at convenient temperatures and that ^{195}Pt NMR spectroscopy is uniquely suited to the task. To our knowledge, there have been only two reports on variable-temperature ^{195}Pt NMR spectroscopy;[13] this seems to be the third one and the second in which NMR spectroscopy of this nucleus is applied to a problem of stereodynamics. We undertook this study in spite of the recently expressed skepticism regarding the suitability of the ^{195}Pt nucleus for variable-temperature experiments.[10] We believe that metal nuclei[14-17] are certain to find use in dynamic NMR spectroscopy.

Experimental Section

Materials. Chemicals *N*-acetyl-L-methionine and *N*-acetyl-D-methionine were obtained from Sigma Chemical Co.; K_2PtCl_4 was obtained from Aldrich Chemical Co. and borrowed from Johnson Matthey, Inc. Absorption spectra were recorded with an IBM 9430 spectrophotometer, equipped with a two-grating monochromator.

NMR Measurements. The ^1H and ^{13}C (at 74.5 MHz) NMR spectra were recoreded with JEOL FX 90 Q and Nicolet NT 300 spectrometers, using residual H_2O and dioxane as the respective internal standards. The ^{195}Pt NMR spectra of unenriched samples were recorded with a Bruker WM 300 spectrometer at 64.4 MHz, using a 10-mm probe. Each spectrum was acquired in 8K data points, with both of the following sets of parameters corresponding respectively to the spectral width, pulse duration, tilt angle, acquisition time, and delay time: 100 kHz, 10 μs, 13°, 51 ms, 200 ms; 20 kHz, 65 μs, 90°, 205 ms, 3300 ms. The sample temperature was maintained within ±0.5 deg using the Bruker variable-temperature controller. The spectrum at 365 K is somewhat noisier than those at lower temperatures. Solution of K_2PtCl_4 in aqueous NaCl, kept in a coaxial inset tube, was used as an external reference. The ^{195}Pt chemical shifts at 21 °C with respect to the $PtCl_6^{2-}$ standard can be obtained by subtracting 1614 ppm from the corresponding values with respect to the $PtCl_4^{2-}$ reference. Signals occurring at stronger fields than the reference signal have negative chemical shifts. Since the $PtCl_4^{2-}$ and $PtCl_6^{2-}$ ions have similar compositions and identical charges, their chemical shifts depend similarly on temperature. In view of the small magnitude of these temperature effects and of the extremely wide range of ^{195}Pt chemical shifts, the use of the same correction factor at other temperatures would have little, if any, effect on the discussion and conclusions.

Table I. ^{13}C NMR Chemical Shifts, in ppm Downfield from Me_4Si

atom	free AcMetH[a]	$[Pt(AcMetH)Cl_3]^-$
C^5	14.1	20.8
C^7	21.7	22.2
C^3	29.4	28.5
C^4	29.9	34.8
C^2	51.7	51.7
C^6	174.2	174.3
C^1	175.4	174.6

[a] *N*-acetyl-L-methionine.

Synthesis of K[Pt(AcMetH)Cl$_3$]. A solution of 95 mg (0.5 mmol) of *N*-acetyl-L-methionine in 1.5 mL of water was added dropwise, with stirring, to a solution of 208 mg (0.5 mmol) of K_2PtCl_4 in 1.5 mL of water. The reaction was best carried out in the dark, to minimize formation of Pt metal and other side reactions; the use of dilute (ca. 0.25 M) HCl instead of pure water as a solvent also contributed to the suppression of side reactions. The color change from reddish to brownish yellow was virtually complete within 2 h at room temperature. The solution was kept at 50 °C for 8 h and filtered to remove any particles. Separate experiments in which the reaction was followed by ^1H NMR and UV–vis spectroscopy confirmed that under those conditions the substitution is complete. The compound was not isolated.

The title compound was unaffected by prolonged heating at 95 °C. The samples for NMR measurements usually were prepared in D_2O; those prepared in H_2O were alternately desiccated and dissolved in D_2O before spectra were recorded. The samples prepared in these two ways yielded identical spectra.

Results and Discussion

Substitution Reaction and Spectra. The substitution of AcMetH for a Cl^- ligand is accompanied by the disappearance of the weak bands at 392 and 331 nm, due to the $PtCl_4^{2-}$ starting complex, from the absorption spectrum of the reaction mixture. The remaining strong ultraviolet absorption represents the featureless tail of a band whose maximum occurs below 190 nm. The changes in the ^1H and ^{13}C NMR spectra of AcMetH upon coordination are simple and informative. The free AcMetH ligand exhibits two methyl singlets in its ^1H spectrum: that of CH_3S at 1.96 ppm and that of $CH_3C(O)$ at 1.89 ppm. In complex **1**, the former signal shifts by 0.31 ppm downfield (to 2.27 ppm) and splits into a 1:4:1 pattern owing to the coupling with ^{195}Pt, whose natural abundance is 34%. The coupling constant ($^3J_{Pt-H}$ = 49.6 Hz) agrees fully with the values reported for complexes containing trans Cl–Pt^{II}–$S(CH_3)_2$ fragments.[18-20] This coupling is evident in the ^1H NMR spectra recorded at 60 and 90 MHz, but in a strong field (at 300 MHz) the relaxation of ^{195}Pt owing to the chemical shift anisotropy causes the satellites to be broadened beyond detection.[21] When the formation of **1** is completed, the singlet of uncoordinated CH_3S group is completely gone, while that of the acetyl group remains unperturbed by coordination. A very small signal at 2.45 ppm indicates the presence of a small amount of *cis*-$[Pt(AcMetH)_2Cl_2]$ in the solution of **1**.

The ^{13}C NMR spectra of the free AcMetH and of **1** at 21 °C show seven signals each, as expected. The chemical shifts are listed in Table I. The fact that only the signals due to atoms C^5 and C^4 are moved by more than 0.9 ppm upon coordination proves that AcMetH is bonded to Pt as a monodentate thioether ligand. Since coordination of transition metals to the deprotonated nitrogen in the amide or peptide bond is a subject of considerable current interest[22] and since very few such complexes of platinum

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have been reported,[23-26] we examined this possibility in the case of the ligand AcMetH. The ^{13}C chemical shifts of C^6 and C^7 atoms are unaffected by coordination to platinum (Table I), and the pH value remains constant during the substitution reaction. Evidently, the amide N atom remains protonated. This finding is relevant to the study of $PtCl_4^{2-}$ reactions with methionine in proteins, because their peptide bonds must always be considered as potential binding sites for metals. Although the thioether ligand is an effective "anchor",[22] the product of chelation involving the amide N atom would be a six-membered ring. A survey of the literature[23-26] indicates that the coordination of the amide nitrogen to Pt(II) is facilitated if it yields a five-membered ring or a larger chelate containing such a ring.

Variable-Temperature ^{195}Pt NMR Spectroscopy. Since the chiral C^2 atom in L-methionine is stable toward racemization under the conditions of our experiments, chirality of the S atom in **1** gives rise to two diastereomers, **1a** and **1b**. The ^{1}H and ^{13}C NMR

1a **1b**

spectra at room temperature give no evidence of these two species. In particular, the respective signals of the CH_3 substituent at the S atom show no doubling at room temperature. Evidently, the chemical shifts of ^{13}C and ^{1}H nuclei are insufficiently sensitive to their respective environments to reflect the subtle difference between **1a** and **1b**. The smaller the frequency difference $\Delta\nu$ between the signals due to the isomers, the lower the temperature at which the signals will coalesce as the isomers interconvert.[27-30] In this case the difference between the ^{13}C signals or the ^{1}H signals due to **1a** and **1b** is so small that the signals are already averaged at 278 K, the lowest temperature we used. Since the known ^{195}Pt chemical shifts span a range of some 15 000 ppm,[15] we applied ^{195}Pt NMR to this case. As Figure 1 shows, the spectrum at 278 K consists of two signals of equal intensities, with chemical shifts of −1168 and −1174 ppm with respect to the $PtCl_4^{2-}$ reference; they correspond to −2782 and −2788 ppm, respectively, on the scale based on the $PtCl_6^{2-}$ standard, as explained in the Experimental Section. These values agree with the value of −2757 ppm, observed for the simple thioether complex $[PtCl_3(SMe_2)]^-$.[31,32] The broad lines in the strong magnetic field result, in part, from chemical shift anisotropy, as discussed above. In view of the great dependence of the ^{195}Pt chemical shifts on the donor atom,[31] the two signals differing by only 6 ppm (or 366 Hz) cannot be due to complexes of different compositions; they represent the diastereomers **1a** and **1b**. The ^{195}Pt spectrum of complex **1** containing *N*-acetyl-D-methionine is indistinguishable from the spectrum of the compound containing the L enantiomer of the ligand, as expected.

The proof of the assignment of the two ^{195}Pt signals lies in their coalescence and the noticeable, although not pronounced, sharpening of the averaged signal upon heating above the coalescence temperature. The spectra were recorded at nine different temperatures from 278 to 364 K, spanning the range accessible in water; the four characteristic ones are shown in Figure 1. All the temperature-related changes are reversible. The same results were obtained with 0.10 and 0.30 M sample solutions and under two different sets of acquisition conditions, specified in the Experimental Section. The small and uniform movement of the signals upon heating agrees with the known temperature dependence of the ^{195}Pt chemical shift in similar compounds.[31,32]

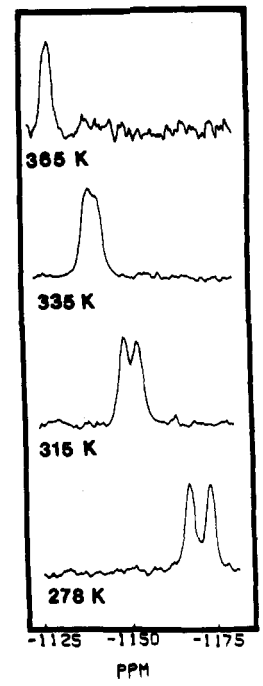

Figure 1. ^{195}Pt NMR spectra of 0.30 M $K[Pt(AcMetH)Cl_3]$ in H_2O, recorded at the four temperatures indicated. The coalescence temperature is 335 K. The chemical shifts are referenced to $PtCl_4^{2-}$ ion, for which the spectrum reference (SR) of 63 695 Hz has been set at 278 K.

Diastereomers **1a** and **1b** furnish the simplest example of an interchange process, the one represented by two singlets of equal intensities merging into a single band. Formulas that are often used approximately in the more complicated cases actually apply to this one[27-30] and yield the exchange rate constant k of 785 s^{-1} and the corresponding activation energy $\Delta G^{\ddagger}$ of 63.7 kJ mol^{-1} at the coalescence temperature of 335 K. Not only is ^{195}Pt NMR the only method applicable to the study of stereodynamics in **1** and similar compounds, the resulting spectrum is so simple that the method is applicable, in principle, to complex natural products, including the macromolecular ones. Even compounds that are less thermally stable than **1**, and those that are more fluxional than **1**, can be studied by ^{195}Pt NMR if a suitable magnetic field is used. For example, the coalescence temperatures for **1** at 2.11 and 11.7 T, corresponding respectively to 90 and 500 MHz for ^{1}H, would be about 317 and 343 K, respectively.[27-30]

Sulfur Inversion. The spectra in Figure 1 may, in principle, be explained in terms of several mechanisms. (1) Since the value of $\Delta G^{\ddagger}$ is relatively large, the substituents on the S atom are rather heavy, and species **1a** and **1b** are diastereomers, nuclear tunneling can be ruled out.[10] (2) Dissociation of one of these substituents and subsequent recombination can also be ruled out. This mechanism would require an activation energy far in excess of the value obtained and would not permit the sharpening of the ^{195}Pt NMR signal above the coalescence temperature, which is discernible in Figure 1. Other Pt(II)–thioether complexes were shown to maintain the ^{1}H–^{195}Pt coupling above the coalescence temperature[10,33] and not to exchange the thioether ligands.[10,34]

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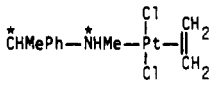
(3) Since we observed the same coalescence temperature and thus obtained the same values of k and ΔG^*, with the 0.10 and 0.30 M solutions of **1**, bimolecular exchange can also be ruled out.[10]
(4) We conclude that the temperature dependence of the ^{195}Pt NMR pattern is caused by intramolecular inversion of configuration at the chiral S atom. Indeed, our value of ΔG^* falls in the middle of the rather narrow range of such values obtained for Pt(II) complexes with common thioether ligands.[10]

Diastereomeric Discrimination. Relatively little is known about the dependence of transition-metal chemical shifts on subtle changes in the molecular structure.[14-17,31] Since stereoselectivity and regioselectivity in chemical reactions often arise from such subtle changes and since the ^{195}Pt nucleus evidently can sense them, we are interested in the origins of the 6-ppm difference between the ^{195}Pt NMR signals of species **1a** and **1b**. To our knowledge, there are only two compounds, designated **2**[35] and **3**,[36] for which



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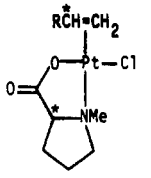

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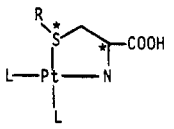
the ^{195}Pt chemical shift was shown to be sensitive to diastereomerism; the chelating ligand in **3** is *N*-methyl-L-proline. Several other complexes are relevant to this study. Formula **4** represents



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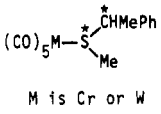
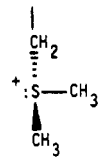


M is Cr or W

5

several *S*-alkylcysteine chelates,[37-40] for which no ^{195}Pt NMR spectra have been reported. The compound designated **5**, although not containing platinum, is related to our complex **1** in that it contains a chiral monodentate thioether ligand.[41] (Needless to say, various other platinum complexes exhibit diastereomerism.) Diastereomerism of all the compounds **2** through **5** is clearly evident in their ^1H and ^{13}C NMR spectra at room temperature, but that of **1** is evident only in its ^{195}Pt spectrum. Complex **1** is also the only one whose two diastereomers are virtually equally abundant. This weak diastereomeric discrimination between **1a** and **1b** permits, in principle, an investigation into the details of molecular structure to which the ^1H and ^{13}C nuclei are not sensitive but the ^{195}Pt nucleus is. Since our attempts to obtain single crystals of salts containing anion **1** have been unsuccessful, the discussion of its expected structure will rest upon the known structures of related compounds.

The molecular structures are known for two methionine derivatives containing chiral sulfur atoms: *S*-methyl-L-methionine chloride[42] and L-methionine sulfoximine,[43] whose relevant fragments are designated **6** and **7**, respectively; the rest of each



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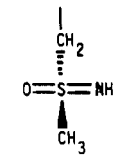

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molecule is the same as in formula **1**. In both of these derivatives, the side chain adopts a nearly planar, trans conformation, i.e., an extended zigzag shape. Acylation of the amino group and esterification of the carboxyl group in methionine do not seem to affect the conformation of the side chain—both *N*-formyl-L-methionine[44] and *N*-acetyl-L-methionine methyl ester[45] have their respective side chains nearly in the fully extended conformation. This conformational preference may be small, perhaps arising from the packing forces and hydrogen bonding in the crystals. For example, solid methionine exists in two forms, which differ from each other only in the position of the CH_3S group with respect to the rest of the molecule, in particular to the C^3 methylene group. In the trans form, the side chain is fully extended, with the C^4–S dihedral angle of 186°; in the gauche form, this angle is 69°.[46,47] A theoretical analysis of the conformation of **6** indicated this molecule to be highly flexible. Considerations of biological activity and of NMR spectra of **7** indicated that in solution this molecule adopts an extended conformation, with the chiral atoms S and C^2 as far apart as possible.[43]

The side chain in the complex **1** probably also adopts an essentially trans planar conformation in solution. Although the S atom bears a formal positive charge, its approach to the COO^- group, which seems possible for compound **6**,[42] would be hindered by the bulk and charge of the $PtCl_3^-$ substituent. Indeed, binding of platinum to the S atom has an insignificant effect upon the ^{13}C chemical shift of the C^1 atom (Table I). In both compounds **6** and **7**, as well as in free methionine, the C^5–S bond is slightly shorter (by ca. 0.02 Å) than the C^4–S bond. This difference in bond lengths perhaps explains why coordination of the Pt(II) complex to the S atom causes a slightly greater downfield shift of the C^5 peak than of the C^4 peak in the ^{13}C NMR spectrum of AcMetH (Table I). Although the presumed extended conformation of the side chain in **1** maximizes the distance between the Pt atom and the polar moiety (the backbone) of the AcMetH ligand, molecular models show that the Pt atom has different environments in diastereomers **1a** and **1b**, particularly with respect to the COO^- and C(O)NH groups. The 6-ppm difference between the ^{195}Pt NMR signals of the diastereomers probably is caused by long-range interactions of the metal atom with these two groups. It is difficult to judge the relative importance of the direct and indirect (through the Cl^- ligands) influences on the Pt atom. The fact that the diastereomers are nearly equally abundant indicates that the chiral C^2 atom provides virtually no discrimination between the two configurations at the S atom.

Conclusion

We have shown that variable-temperature ^{195}Pt NMR spectroscopy can be readily applied in stereodynamic experiments. In particular, it can be used to study dynamic processes causing subtle changes in the molecular structure and those involving relatively complex biomolecules. Such processes, one of which is examined in this report, are not easily tractable by the common ^1H and ^{13}C NMR methods. Although the ^{195}Pt nucleus is less receptive than ^1H, it is 19.1 times more receptive than ^{13}C and should permit spectroscopic measurements even with relatively dilute solutions. The enhancement of chemical shift anisotropy upon binding of the Pt atom to a macromolecule, however, will facilitate relaxation

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and render the NMR signals difficult to detect.[5] This problem notwithstanding, ^{195}Pt NMR spectroscopy offers important advantages for the study of complex natural products. Since the ^{195}Pt chemical shifts span a range greater than that of any other nucleus, they are highly sensitive to the nature of the ligands and, as this and other studies showed, even to the subtle changes in the molecular environment. Labeling of different groups on a biomolecule, and even of similar groups differently located, should give rise to well-separated ^{195}Pt NMR signals. We are working to develop the $PtCl_3^-$ complex and its derivatives into NMR labels for the studies of structural and dynamic properties of proteins.

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Contribution from the Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada

Nuclear Magnetic Resonance Studies of the Solution Chemistry of Metal Complexes. 23. Complexation of Methylmercury by Selenohydryl-Containing Amino Acids and Related Molecules

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The acid–base chemistry and the methylmercury(II) (CH_3Hg^{II}) complexation chemistry of selenoacetic, selenopropionic, and selenobutyric acids, selenocysteine, selenocysteamine, selenopenicillamine, and 2-hydroxyethaneselenol have been studied in D_2O solution. Aqueous solutions of the selenohydryl ligands were prepared by reduction of diselenides at a mercury-pool cathode. Acid dissociation constants for the ligands were determined by pH titration, and those for the CH_3Hg^{II}–selenol complexes were determined from ^1H NMR chemical shift data. Equilibrium constants for displacement of mercaptoacetic acid from its CH_3Hg^{II} complex by the selenol ligands were obtained from ^1H NMR chemical shift data for the mercaptoacetic acid. Formation constants for the CH_3Hg^{II}–selenol complexes were calculated from the displacement constants and the formation constant of the CH_3Hg^{II}–mercaptoacetic acid complex. Formation constants for the CH_3Hg^{II}–selenol complexes are 0.1–1.2 log K units larger than those for the corresponding thiol complexes. Despite the large formation constants, exchange of CH_3Hg^{II} between selenol ligands, and between selenol and thiol ligands, is fast on the ^1H NMR time scale. The possibility of in vivo complexation of CH_3Hg^{II} by selenocysteinyl sites of glutathione peroxidase is discussed.

Introduction

Since the outbreak of Minimata disease in Japan in the 1950s, there has been widespread interest in the biological chemistry of methylmercury(II) (CH_3Hg^{II}). CH_3Hg^{II} has such a high affinity for sulfur that it is assumed to be all complexed by thiol groups of amino acids, peptides, and proteins in vivo.[1-3] Direct experimental evidence for thiol complexation has been obtained from ^1H NMR studies of CH_3Hg^{II}-containing intact human erythrocytes.[4] These ^1H NMR studies also show that, despite the large formation constants of the CH_3Hg^{II}–thiol complexes,[3,5-7] CH_3Hg^{II} exchanges rapidly among thiol ligands in intact cells.[4,7-9]

An intriguing aspect of CH_3Hg^{II} toxicology is that various selenium-containing compounds have significant protective effects in animal studies of CH_3Hg^{II} poisoning.[10,11] The molecular basis for this effect has not yet been established; however, it is possible that it involves complexation of CH_3Hg^{II} by selenium donor groups. The complexation of CH_3Hg^{II} by selenium is also of interest since selenium is present, as a selenolate anion, at the active site of glutathione peroxidase.[12] If CH_3Hg^{II}–selenolate complexes are more stable than CH_3Hg^{II}–thiolate complexes, and if exchange of CH_3Hg^{II} between thiolate and selenolate groups is sufficiently fast, a significant fraction of the enzyme would be CH_3Hg^{II}-complexed, which presumably would affect its ability to protect cells from peroxidative damage.[13]

Since selenium is softer in a Lewis base sense than sulfur, it would be expected to form more stable complexes with class B metal ions such as CH_3Hg^{II}.[14] In support of this, the formation constant of $CH_3HgSeCN$ is larger than that of CH_3HgSCN[15] and the ^{199}Hg–^1H coupling constant, which decreases as the formation constant increases,[3] is smaller for the CH_3Hg^{II} complexes of Se^{2-}, $^+NH_3CH_2CH_2Se^-$, CH_3Se^-, and $PhSe^-$ than for the complexes of the sulfur analogues.[15-17] Structural data also suggest that the Hg–Se binding in $CH_3HgSeCH_2CH(NH_3)CO_2 \cdot H_2O$ is stronger than the Hg–S binding in the analogous cysteine complex.[18] However, formation constants with which the relative strengths of CH_3Hg–Se and CH_3Hg–S binding can be quantitatively compared have not been reported.

In this paper, we present the results of ^1H NMR studies of the

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