ADDUCTS OF ETHYLMERCURY PHOSPHATE WITH AMINO ACIDS STUDIED BY INDIRECT DETECTION OF ^{199}Hg NMR

Mary F. ROBERTS*, David A. VIDUSEK* and Geoffrey BODENHAUSEN+

Department of Chemistry and +*Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139, USA*

Received 14 May 1980

1. Introduction

Organomercury compounds are known for their toxic effects, which are often attributed to the formation of covalent mercury–sulfur bonds with cysteine groups in proteins. Ethylmercury phosphate (EMP) is commonly used to form heavy atom derivatives of proteins for X-ray crystallography. Although bonding to accessible cysteine residues tends to be preferred [1], X-ray studies indicate that EMP can also interact with histidine and tryptophan groups [2]. This could explain why EMP inactivates cobra venom phospholipase A_2 [3], which has no free cysteine residues but does contain a reactive histidine group [4]. The mercury–nitrogen bond is readily cleaved in amino acid analysis and cannot be detected by chemical assays. In the absence of extensive X-ray information about the binding of EMP to various amino acids, we have turned to ^{199}Hg NMR to explore the effects of EMP in solution. Preliminary results indicate that the chemical shifts are characteristic for various adducts.

2. Materials and methods

Equimolar mixtures of EMP with various amino acids and related compounds were prepared in volumes and concentrations typical for proton NMR at 270 MHz, i.e., 0.4 ml 10–100 mM solutions. ^{199}Hg ($I = 1/2$) has a natural abundance of 16.9% and a sensitivity of $(48.3/270)^{5/2} = 1.4\%$ compared to an equal number of protons. Although the direct observation by ^{199}Hg Fourier transform NMR is feasible with large, concentrated samples [5,6], better sensitivity can be achieved if the ^{199}Hg resonances are detected indirectly through proton NMR. In ethylmercury derivatives, the scalar coupling $^3J_{HHg}$ to the methyl protons (250–350 Hz) leads to well-resolved satellites in the proton spectrum and can be used to transfer spectral information between the mercury and the proton transitions. Instead of using Indor [7] or CW decoupling methods, which tend to be tedious because the ^{199}Hg chemical shifts extend over some 2500 ppm [6], it is possible to use strong RF pulses to cover a broad spectral window in the vicinity of the ^{199}Hg Larmor frequency.

A rough estimate of the mercury resonance frequency can be obtained by a spin-echo technique derived from [8]. The proton spectrum is obtained by Fourier transformation of a spin-echo generated by a $90°-\tau-180°-\tau$–acquisition sequence, the interval τ being set to $\sim 1/2$ of the inverse coupling constant. A 180° pulse is applied near the mercury Larmor frequency at the time of the proton refocussing pulse. If this ^{199}Hg pulse is on resonance, the spins are inverted and the scalar modulation of the echo causes the satellites to appear with inverted phases (fig.1). Even with a moderate pulse strength ($\gamma_{Hg}B_1 =$ 2 kHz), it is possible to search for the mercury resonance in increments of 5 kHz (100 ppm). Smaller steps allow the determination of the ^{199}Hg shift to ±500 Hz (10 ppm) by inspection of the partial inversion of the satellites.

The measurement of the chemical shift of ^{199}Hg can be refined by a sensitivity enhanced variation [9] of heteronuclear two-dimensional spectroscopy [10–13]. This method combines the advantage of polarization transfer from protons to mercury with the sensitivity of proton detection. The magnetization is transferred twice, first from the ^{199}Hg satellites in the proton spectrum to the mercury transitions, then back to the proton spectrum. The methyl

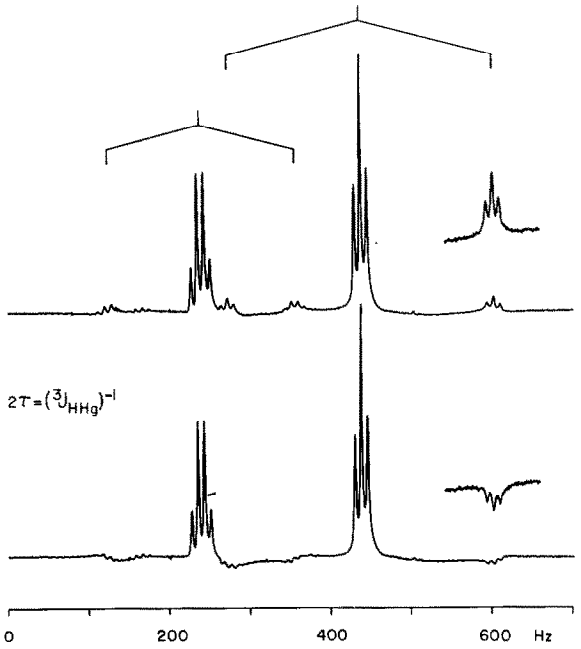

Fig.1. Proton spectra (non-spinning) of ethylmercury phosphate (EMP 155 mM in ^2H_2O at pH 3.2) obtained at 270 MHz by Fourier transformation of a spin echo excited by a $90°-\tau-180°-\tau$ sequence, with $\tau = (2^3J_{HHg})^{-1} = 1.52$ ms. A 180° pulse (140 μs) is applied in synchronism with the refocussing pulse in the ^{199}Hg region. In the top spectrum, the mercury pulse is ~20 kHz off-resonance, and the satellites appear with normal phases. In the second case, the mercury spins are inverted by the on-resonance pulse and the satellites appear upside down. Each spectrum was obtained with 20 transients.

satellites in the proton spectrum are monitored as a function of the ^{199}Hg precession time, generating point by point a time domain signal which is in many respects equivalent to a proton-decoupled mercury free induction decay (fig.2). The sensitivity is determined by the need to observe the satellites in the proton spectrum, and does not depend on the gyromagnetic ratio of the ^{199}Hg nucleus. The technique has been detailed for a pair of spin-1/2 nuclei in [9]. Here, the magnetization is transferred through the 3-bond coupling in the AM_2X_3 system, where the sensitivity is further enhanced by the degeneracy of the 3 methyl protons. The smaller homonuclear MX coupling does not substantially affect the polarization transfer. The maximum theoretical sensitivity advantage of indirect detection methods is an ~200-fold enhancement.

The mercury signal shown in fig.2 provides an

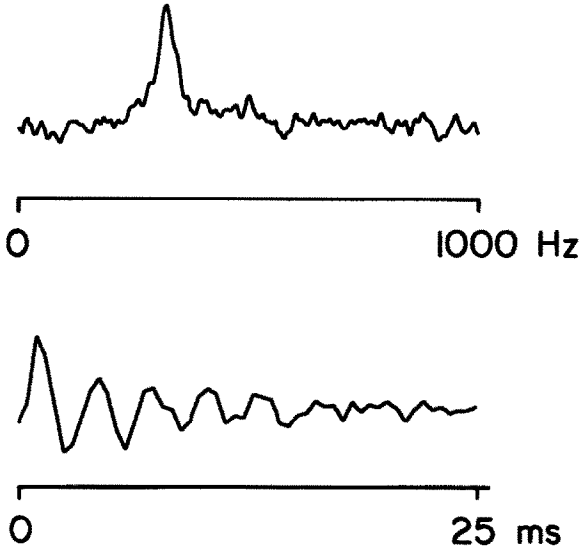

Fig.2. ^{199}Hg spectrum (top) obtained by Fourier transformation of the 'free induction decay' (below), constructed point-by-point by monitoring the amplitude of the ^{199}Hg-satellites in the methyl proton spectrum in the course of a heteronuclear two-dimensional experiment. The origin of the frequency domain $F_1 = 0$ corresponds to the carrier frequency of the mercury pulses.

accurate measure of the transverse relaxation, in direct analogy to an ordinary free induction decay. The inhomogeneous line broadening, which is proportional to the gyromagnetic ratio, can be safely neglected. The technique is suitable for the detection of very broad lines, since the increments and initial duration of the mercury precession time can in principle be made arbitrarily small. The problem of receiver recovery time, which limits the direct observation of fast-decaying free induction signals, is entirely circumvented. The mercury spin-lattice relaxation on the other hand should not be too fast, since the satellites in the proton spectrum must be resolved.

3. Results and discussion

The chemical shifts and linewidths of some adducts are listed in table 1. Both the chemical shift and the linewidth of free EMP are pH-dependent, presumably because of chemical exchange between $CH_3CH_2Hg^+$, $(CH_3CH_2Hg)_2OH^+$ and CH_3CH_2HgOH, in analogy to the methyl compounds [14]. The shifts in table 1

Table 1
^{199}Hg Chemical shifts and linewidths of ethyl mercury adducts

Adduct[a]	pH	^{199}Hg Chemical Shift[b] (ppm)	^{199}Hg Linewidth[c] (Hz)
EMP	3.2	0	10 ± 1
EMP	7.1	31.4 ± 0.4	44 ± 7
EM-Imidazole	8.2	191.1 ± 0.4	53 ± 6
EM-Histidine	5.6	189.2 ± 0.8	277 ± 14
EM-Tryptophan	3.1	256 ± 10	–
EM-Glycine	3.8	7 ± 10	–
EM-Glycine	7.5	231 ± 10	–
EM-Aspartate	2.6	−3 ± 10	–
EM-Aspartate	8.6	38 ± 10	–
EM-3′,5′-c-AMP	7.2	49 ± 10	–
EM-3′,5′-c-GMP	7.4	162 ± 10	–
EM-Cysteine	2.6	566 ± 10	–
EM-2-mercaptoethanol	7.3	608 ± 10	–
EM-thiosalicylate	8.5	500.1 ± 0.1	32 ± 3

[a] Concentrations vary from 10–160 mM
[b] Reference is 155 mM EMP in ^2H_2O (pH 3.2), 295 K. Actual ^{199}Hg resonance at 48, 297, 652 ± 11 Hz in 270 MHz spectrometer
[c] No linewidth indicated if shift determined by spin-echo method only

differ by some 300 ppm between Hg–N and Hg–S adducts, enough to decide by the simple spin-echo technique alone whether EMP is bound to cysteine or to histidine and tryptophan residues in a protein. The tryptophan adduct is shifted by some 66 ppm with respect to the histidine complex.

In the systems considered here, the linewidths provide a measure of chemical exchange. The bonding of EMP to amino acids is associated with a small change in $^3J_{HHg}$ of a few tens of Hz. The exchange is too fast to allow the resolution of separate satellites in the proton spectrum, although some broadening of the satellites could be observed in many systems. The mercury shift however may change by as much as 500 ppm (25 kHz). In some cases, such as the glycine adduct at pH 6, the exchange is sufficiently slow to detect two separate mercury resonances by the spin-echo technique, one due to free EMP and the other due to the nitrogen complex. In intermediate cases, the mercury linewidth appears to be in excess of 500 Hz, and cannot be measured adequately with the slow, computer-controlled pulse program used in these experiments. Broad lines have also been reported for ionic mercury bound to a metalloenzyme [15]. In addition to chemical exchange, the mercury resonance may be broadened by scalar relaxation due to coupling to the fast-relaxing quadrupolar ^{14}N nucleus. The ^{199}Hg linewidth should be proportional to the square of the scalar coupling constant J_{NHg}, which could be determined by applying the same technique to ^{15}N-enriched adducts.

In conclusion, ^{199}Hg NMR appears a useful probe to study the interaction of mercury compounds with proteins. The enhancement of the sensitivity obtained by indirect detection makes it possible to use low concentrations and small sample volumes, which is highly desirable in biological systems.

Acknowledgements

EMP was the gift of Dr G. A. Petsko of the Department of Chemistry, MIT. The authors are indebted to Dr D. J. Ruben for valuable assistance with the instrumentation. The high field NMR experiments were performed at the NMR facility for Biomolecular Research located at the F. Bitter National Magnet Laboratory, MIT. The NMR facility is supported by grant no. RR00995 from the Division of Research Resources of the NIH and by the National Science Foundation under contract no. C-670. Support from National Institutes of Health, GM 26762 to M.F.R. is also gratefully acknowledged.

References

[1] Banner, D. W., Bloomer, A. C., Petsko, G. A., Phillips, D. C., Pogson, C. I. and Wilson, I. A. (1975) Nature 255, 609.
[2] Tsernoglou, D. and Petsko, G. A. (1976) FEBS Lett. 68, 1.
[3] Koziski, K. and Roberts, M. F. (1980) in preparation.
[4] Roberts, M. F., Deems, R. A., Mincey, T. C. and Dennis, E. A. (1977) J. Biol. Chem. 252, 2405.
[5] Maciel, G. E. and Borzo, M. (1973) J. Magn. Reson. 10, 388.
[6] Sens, M. A., Wilson, N. K., Ellis, P. D. and Odom, J. D. (1975) J. Magn. Reson. 19, 323.
[7] Anet, F. A. L. and Sudmeier, J. L. (1969) J. Magn. Reson. 1, 124.
[8] Emshwiller, M., Hahn, E. L. and Kaplan, D. (1960) Phys. Rev. 118, 414.
[9] Bodenhausen, G. and Ruben, D. J. (1980) Chem. Phys. Lett. 69, 185.
[10] Maudsley, A. A. and Ernst, R. R. (1977) Chem. Phys. Lett. 50, 368.
[11] Maudsley, A. A., Müller, L. and Ernst, R. R. (1977) J. Magn. Reson. 28, 463.
[12] Bodenhausen, G. and Freeman, R. (1977) J. Magn. Reson, 28, 471.
[13] Müller, L. (1979) J. Am. Chem. Soc. 101, 4481.
[14] Sudmeier, J. L., Birge, R. R. and Perkins, T. G. (1978) J. Magn. Reson. 30, 491.
[15] Sudmeier, J. L. and Perkins, T. G. (1977) J. Am. Chem. Soc. 99, 7732.