One might argue that the outstandingly high activity of the pellet form of Ca_6-A could be due to the binder in the pellet, which might have been activated by the ion exchange. However, this may be explained by the fact that the bead-form Ca_6-A, containing the same binder, showed the same activity at 150 °C as that of powder-form Ca_6-A which contained no binder. Thus, it is concluded that the high catalytic activity of Ca_6-A is attributed to Ca^{2+} on the α site.

The powder-, pellet-, and bead-form catalysts exhibited considerably different activities. These may be attributed to their texture or pluggings of the zeolite surfaces, which influence the diffusion of the reactants and products. Hence such results do not affect the above conclusion.

References and Notes

(1) T. Takaishi, Y. Yatsurugi, A. Yusa, and T. Kuratomi, *J. Chem. Soc., Faraday Trans. 1*, **71**, 97 (1975); K. Seff, *Acc. Chem. Res.*, **9**, 121 (1976); D. W. Breck, "Zeolite Molecular Sieves", Wiley, New York, N.Y., 1974; and papers quoted in these.
(2) T. Ohgushi, A. Yusa, and T. Takaishi, *J. Chem. Soc., Faraday Trans. 1*, in press.
(3) R. Firor and K. Seff, *J. Am. Chem. Soc.*, submitted for publication.
(4) P. C. W. Leung, K. B. Kunz, K. Seff, and I. E. Maxwell, *J. Phys. Chem.*, **79**, 2157 (1975).

T. Takaishi*
Institute for Atomic Energy, Rikkyo University
Yokosuka, Japan 240-01

H. Hattori
Department of Chemistry, Hokkaido University
Sapporo, Japan 060

Received July 14, 1977

Studies of Single $^{199}Hg^{II}$ Ion Resonances in the Active Site of Human Carbonic Anhydrase B by Fourier Transform Nuclear Magnetic Resonance

Sir:

We wish to report the first direct observation by NMR of $^{199}Hg^{II}$ resonances from a Hg^{II}-protein complex in solution. The very strong, specific binding of Hg^{II} and organomercurials to many proteins, especially those having exposed sulfhydryl groups, has long been employed to provide heavy atom labels in protein x-ray crystallography.[1] The powerful enzyme-inhibiting properties of mercury and its compounds accounts for their well-known toxicities to all living organisms.[2] In the present work we have substituted $^{199}Hg^{II}$ for the naturally occuring Zn^{II} in the active site of the zinc metalloenzyme human carbonic anhydrase B (carbonate hydro-lyase EC 4.2.1.1).[3]

Hg^{II} binds more tightly to HCAB than any other metal ion, but has failed to restore significant activity to carbonic anhydrases under conditions employed to now.[4] Nevertheless, the spin quantum number, $I = 1/2$ and extremely large chemical shift range of known ^{199}Hg compounds (>2500 ppm)[5-7] makes ^{199}Hg interesting as a possible spectroscopic probe of the active site. Furthermore, in conjunction with ^{113}Cd ($I = 1/2$) NMR studies of zinc enzymes,[8] we may obtain useful extrapolations among the d^{10} metal ions to the properties of the zinc enzyme, which has no common Zn isotope with favorable nuclear spin for NMR studies.

Figure 1A shows the ^{199}Hg NMR spectrum[9] at 25 °C of ~4 mL of 7 mM aqueous $^{199}Hg^{II}HCAB$[10] in 0.05 M Tris acetate buffer at pH 7.7. A single, broad (~400 Hz) peak centered at −1310 ppm upfield of neat $(CH_3)_2Hg$ is consistently observed under various sweepwidth and spectrometer offset conditions. The peak broadness in $^{199}Hg^{II}HCAB$ is analogous to our results for uninhibited $^{113}Cd^{II}HCAB$,[8b] and suggests that both

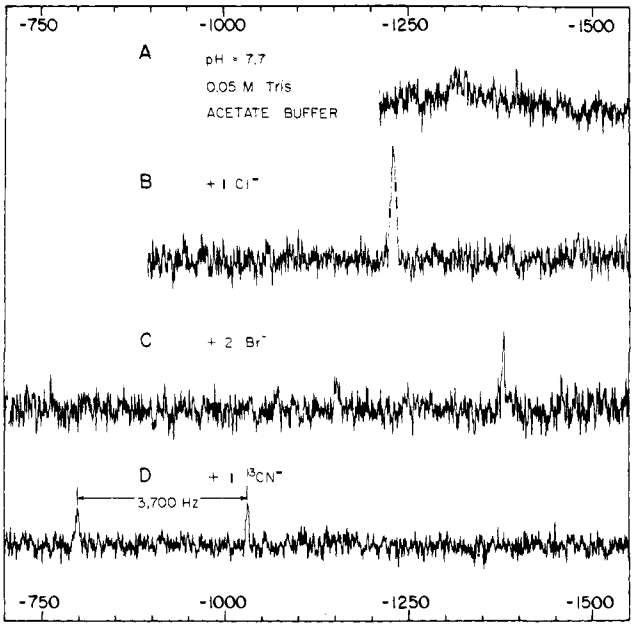


Figure 1. ^{199}Hg FTNMR proton-coupled spectra at 25 °C of 83.5% isotopically enriched 7 mM $^{199}Hg^{II}HCAB$ in 50 mM aqueous Tris acetate, pH 7.7. Chemical shifts (parts per million) are relative to neat $(CH_3)_2Hg$. Exponential multiplication yielding 10-Hz line broadening was applied to the free-induction decays. All spectra are 12-h accumulations: (A) original sample, (B) sample A plus 1 equiv of NaCl, (C) sample B plus 2 equiv of NaBr, (D) sample B plus 1 equiv of $K^{13}CN$ (90% isotopically enriched).

metals undergo ligand-ligand exchange processes at intermediate rates on the NMR time scale. The fact that the Hg^{II} ion occupies the active site of HCAB (rather than the partly accessible sulfhydryl group)[3b] when equimolar Hg^{II} is adduced from the failure of Zn^{II} to restore significant activity.[11]

Figure 1B shows the dramatic change in the ^{199}Hg spectrum caused by addition of 1 equiv of NaCl, analogous to the changes in ^{113}Cd NMR spectra of $^{113}Cd^{II}HCAB$.[8b] The ^{199}Hg resonance shifts downfield (to −1220 ppm) and sharpens to a line width of ~110 Hz or less (sometimes as sharp as ~60 Hz). Evidently Cl^- is strongly bound to or near Hg^{II} in the active site, interrupting the ligand-ligand exchange process. Addition of three equivalents of $NaHCO_3$ has no effect on the spectrum shown in 1B

Figure 1C shows the effect of adding 2 equiv of NaBr to the sample giving rise to Figure 1B. Br^- appears to displace Cl^-, as is also the case in the Cd^{II}[12] and native Zn^{II}[3a] enzymes. The peak at −1380 ppm has a line width of ~50 Hz. In $Cd^{II}HCAB$ containing one Br^- we have evidence[12] for two distinct ^{113}Cd resonances of unequal areas in slow exchange, probably indicating inner sphere and outer sphere binding of Br^-. It is tempting to draw a similar conclusion for $Hg^{II}HCAB$ from the small peak at ~1150 ppm, but until a better signal-to-noise ratio is obtained we reserve judgement.

Figure 1D results from addition of 1 equiv of $K^{13}CN$ (≤90% isotopic enrichment, Merck and Co.). The doublet centered at −910 ppm with a separation of $J_{HgC} = 3700$ Hz and a line width of ~70 Hz indicates direct Hg-C binding. Addition of an additional 3 equiv of $K^{13}CN$ produced no change in the splitting pattern, although J_{HgC} decreased by 40 Hz, presumeably through ionic strength or other medium effects. The tendency of Hg^{II} to bind only one CN^- under these conditions, like the analogous result in $Cd^{II}HCAB$, is surprising in view of its larger ionic radius and consequent displacement (~0.6 Å) away from the three histidyl ligands, at least in crystalline HCAC.[3c]

Preliminary results with ^{15}N-enriched ethylenediamine (en) dihydrochloride indicate that en cannot displace Cl^- from the

active site of Hg^{II}HCAB. Therefore the order of ligand binding strengths observed to date is $CN^- > Br^- > Cl^- > en \approx HCO_3^- \approx CH_3CO_2^-$ in the active site of Hg^{II}HCAB.

Longitudinal relaxation times, T_1, for ^{199}Hg in $^{199}Hg^{II}$-HCAB are ~2 s, based on optimization of the flip angle. The use of broad-band proton decoupling, which was impractical with the homemade probe used herein, should lead to line narrowing and, hence, to improve signal-to-noise ratios in future experiments.

Acknowledgments. This research was initiated under NIH. Special Research Fellowship 1-FO3-GM-54,907-01 (1972-1973) and was supported by NSF Grants GP-38122 and MPS72-05123 A02 and by UCR Intramural Grants. The UCR Bruker WH90D-18 multinuclear FTNMR spectrometer was provided by Bio-medical Sciences Grant 5-S05 RR07010-09 from NIH and NSF Grant MPS75-06138. T.G.P. was supported by a UC President's Fellowship and the NSF Undergraduate Research Program, summer, 1976. We are deeply grateful for assistance from Sven Lindskog, Stuart Bell, Lou Henderson, Toni Keller, Lars Strid, and Professor Bo G. Malmström.

References and Notes

(1) D. Eisenberg in "The Enzymes", Vol. I, 3rd ed, P. D. Boyer, Ed., Academic Press, New York, N.Y., 1970.
(2) L. Fridberg and J. Vostal, Ed., "Mercury in the Environment; an Epidemiological and Toxicological Appraisal", CRC Press, Cleveland, Ohio 1972.
(3) Carbonic anhydrases[3a] from human erythrocytes (HCA) are monomeric enzymes of mol wt ~29 000, each molecule containing a single equivalent of firmly bound Zn^{II} which is required for catalytic activity. X-ray crystallographic studies of the low-activity (HCAB)[3b] and high-activity (HCAC) isozymes reveal the zinc ions near the bottoms of 12–15-Å clefts, coordinated to nitrogen atoms from three histidyl side chains in distorted tetrahedral geometry, with the fourth coordination sites presumably occupied by water molecules or hydroxide ions. (a) Reviewed by S. Lindskog, L. Henderson, K. K. Kannan, A. Liljas, P. O. Nyman, and B. Strandberg in "The Enzymes", Vol. V, 3rd ed, P. D. Boyer, Ed., Academic Press, New York, N.Y., 1971, pp 587–665. (b) K. K. Kannan, B. Notstrand, K. Fridborg, S. Lovgren, A. Ohlsson, and M. Petef, *Proc. Natl. Acad. Sci. U.S.A.*, **72,** 51 (1975). (c) A. Liljas, K. Kannan, P.-C. Bergsten, I. Waara, K. Fridborg, B. Strandberg, U. Carlbom, L. Jarup, S. Lovgren, and M. Petef, *Nature New Biol.*, **235,** 131 (1972); A. Liljas, "Crystal Structure Studies of Human Erythrocyte Carbonic Anhydrase C at High Resolution", Almquist and Wiksell, Stockholm, 1971.
(4) S. Lindskog and P. O. Nyman, *Biochim Biophys. Acta*, **85,** 462 (1964).
(5) G. E. Maciel and M. Borzo, *J Magn. Reson.*, **10,** 388 (1973).
(6) M. A. Sens, N. K. Wilson, P. D. Ellis, and J. D. Odom, *J. Magn. Reson.*, **19,** 323 (1975).
(7) H. Krüger, O. Lutz, A. Nolle, and A. Schwenk, *Z. Phys. A*, **273,** 325 (1975).
(8) (a) I. M. Armitage, R. T. Pajer, A. J. M. Schoot Uiterkemp, J. F. Cheblowski, and J. E. Coleman, *J. Am. Chem. Soc.*, **98,** 5710 (1976); (b) J. L. Sudmeier and S. J. Bell, *ibid.*, in press; (c) D. B. Bailey and P. D. Ellis, 18th Experimental NMR Conference, Asilomar, Calif., April 1977
(9) ^{199}Hg NMR spectra were obtained at ~16.10 MHz on a multinuclear Bruker WH90D-18 using 10- and 15-mm tubes and external ^2H field/frequency lock.
(10) HCA was prepared from erythrocytes by the method of L. E. Henderson and D. Henriksson, *Anal. Biochem.*, **51,** 288 (1973), and apo-HCAB by the method of S. Lindskog and P. O. Nyman.[4] ^{199}Hg HCAB was made by direct addition to a concentrated apoenzyme solution of 1 equiv of ^{199}Hg acetate, the latter prepared by dissolving ^{199}HgO (83.5% enriched, Oak Ridge National Laboratories) in a slight excess of acetic acid followed by neutralizing with Tris.
(11) J. E. Coleman, *J. Biol. Chem.*, **242,** 5212 (1967).
(12) J. L. Sudmeier, unpublished work.

James L. Sudmeier,* Thomas G. Perkins
Department of Chemistry, University of California
Riverside, California 92521
Received June 27, 1977

Preparation of Polymer-Bound Bipyridine and Some of Its Transition Metal Complexes

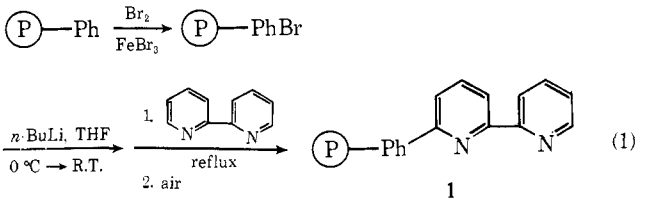
Sir:

Anchoring reagents to insoluble supports has come to be known as solid-phase synthesis. Based on the pioneering efforts of Merrifield in polypeptide synthesis,[1] rapid developments now not only make polypeptide synthesis on polymer supports routine, but immobilized enzymes, immobilized photosensitizers, immobilized organic reagents, and immobilized transition metal catalysts are also frequently reported.[2]

The immobilized transition metal catalyst offers, potentially, a plethora of practical advantages. Significant examples, generally employing phosphine ligands, have been reported by Grubbs,[3] Pittman,[4] Whitehurst,[5] and others.[6,7] Unfortunately, polymeric phosphine transition metal complexes are occasionally so labile that heterogeneous catalysis is not observed[8] or leaching of the metal from the polymer limits catalyst reusibility.[9] In addition, potential applications exist in analysis, in single reactions, in photoprocesses, and in other aspects of synthesis which make these polymer-based reagents of great general potential.

To increase the general availability of immobilized transition metal catalysts, we undertook the synthesis and study of polymer-based chelating ligands.[10] We report herein the facile synthesis of a remarkably general polymer-bound chelating ligand and demonstrate its versatility by the many transition metal complexes prepared from it.

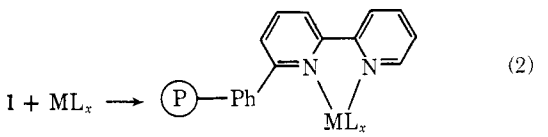
Bipyridine is attached to phenyl residues of polystyrene–2% divinylbenzene copolymer beads through the reaction sequence shown in eq 1. Ring bromination and lithiation are commonly



(1)

used in the preparation of polystyrene derivatives.[16] In our system the stoichiometry of the ring bromination is controlled so that <25% of the phenyl residues are brominated. Solid bipyridine is added to a tetrahydrofuran suspension of the lithiated polymer and the resulting solution is brought to reflux. After a few hours, the solution is allowed to cool to room temperature and air is bubbled through the solution until a color change from dark purple to yellow-gold is observed. The polymer is separated by filtration and washed with copious amounts of various solvents. The yellow-gold polymer, characterized by its infrared spectral data,[17] swells less than the original copolymer in standard solvents and is stable to large quantities of these solvents. Elemental analysis is quantitatively unreliable[18] but suggests ca. one bipyridine per nine styrene units.

Complexation of metal salts with **1** is strikingly facile. In a typical procedure, **1** is added to an $\sim 10^{-3}$ *M* tetrahydrofuran solution of ferric chloride, or other metal salt. The resulting



(2)

solution is shaken for several minutes and the polymer is filtered off and washed with copious amounts of various solvents. The amount of metal bound to the polymer can be determined from the quantitative analysis of the visible spectra of the metal ion solutions before and after exposure to the polymer. The variety of metal salts and the amount of metal incorporated, as determined by this method, are presented in Table I. These data illustrate the generality of this method with respect to metal, oxidation state, and counterion.

The infrared spectra of the metal complexes are very similar to each other and differ from that of **1** by the addition of a