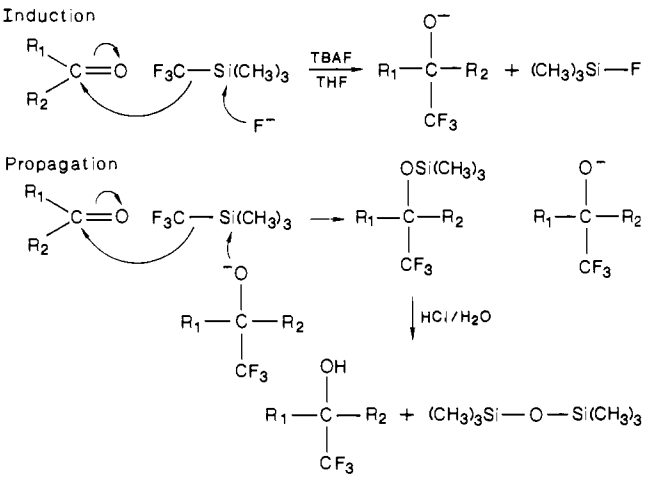
Scheme I



carbonyl compounds and have found a long sought after simple and efficient trifluoromethide equivalent reagent.

Reaction of an equimolar excess of TMS-CF_3 with cyclohexanone under nucleophilic catalysis[15] with a catalytic amount of tetrabutylammonium fluoride (TBAF) in tetrahydrofuran solution generally in an hour gave quantitatively the trifluoromethylated siloxy adduct (i.e., no trace of starting cyclohexanone was observed by GC analysis). In a typical reaction, a mixture of carbonyl compound (10 mmol) and TMS-CF_3 (12 mmol) in 25 mL of tetrahydrofuran cooled to 0 °C in an ice bath is treated with 20 mg of TBAF. Instantaneously a yellow color develops with the initial evolution of fluorotrimethylsilane, and the reaction mixture is brought to ambient temperature and stirred. The mixture is periodically analyzed by GC for the completion of the reaction. The trifluoromethylated siloxy compound is then hydrolyzed to the corresponding alcohol with aqueous HCl.[16] The reaction works equally well with a wide array of aldehydes, ketones, enones, etc. and is generally unaffected by moisture.[17] The obtained yields (of isolated free alcohols) are good to excellent (see Table I). In the case of hindered ketones such as tricyclic 2-adamantanone and tetracyclic estrone methyl ether[18] the reaction is sluggish. Nevertheless, trifluoromethylated adducts were obtained on prolonged stirring (see Table I). In the case of cyclohexenone 1,2-addition predominates (>90%).

Concerning the mechanism, the reaction is induced by fluoride ion (indicated by the irreversible formation of fluorotrimethylsilane in the initial stage of the reaction) and then further catalyzed by the in situ formed trifluoromethylated oxyanion adduct. The reaction also works under alkoxide anion catalysis.[19] The mechanism is depicted in Scheme I.

In conclusion we have developed a versatile new trifluoromethylation method for carbonyl compounds using TMS-CF_3. Further studies are underway to exploit the scope of TMS-CF_3 as a nucleophilic trifluoromethylating agent.

Acknowledgment. Support of our work by the National Science Foundation is gratefully acknowledged.

(15) For an excellent review, see: Furin, G. G.; Vyazankina, O. A.; Gostevsky, B. G.; Vyazankin, N. S. *Tetrahedron* **1988**, *44*, 2675. The reaction did not work under Lewis acid (BF_3:OEt_2, ZnI_2, $TiCl_4$, etc.) catalysis.

(16) The hydrolysis of trimethylsiloxytrifluoromethylated adducts to the corresponding alcohols posed some difficulties. In the case of benzophenone the siloxy adduct was found to be extremely stable.

(17) The tetrabutylammonium fluoride contains three molecules of H_2O, and this did not pose any serious problems in the reaction. However, when used in large quantities under prolonged stirring hydrolysis of the silane is a competing reaction which generates CF_3H. However, in no case generation of difluorocarbene($:CF_2$) was observed.

(18) In the case of sterically hindered estrone methyl ether, more than 1 equiv amount of fluoride was used.

(19) The trifluoromethylation reaction also works equally well when potassium *tert*-butoxide was used as the initiator clearly supporting our proposed mechanism.

^{113}Cd NMR Studies of a 1:1 Cd Adduct with an 18-Residue Finger Peptide from HIV-1 Nucleic Acid Binding Protein, p7

Terri L. South, Bo Kim, and Michael F. Summers*

Chemistry Department
University of Maryland Baltimore County
Baltimore, Maryland 21228

Received August 29, 1988

A central issue in bioinorganic chemistry concerns the relevance and feasibility of zinc binding to putative "zinc fingers" in *RNA-binding* retroviral nucleic acid binding proteins (NABPs).[1] Without exception, retroviral NABPs (and their *gag* precursor proteins) contain the conserved amino acid finger sequence -C-X_2-C-X_4-H-X_4-C- (C = cysteine, H = histidine, X = variable amino acids).[2-4] Although related sequences found in DNA-binding proteins bind Zn^{2+} tightly,[5-10] experiments aimed at measuring the zinc content and the affinity of zinc for retroviral NABPs indicate that zinc binds weakly to these proteins,[11,12] and this has led to the conclusion that zinc is not a structural component of at least one retroviral NABP.[11] On the other hand, recent site-directed mutagenesis experiments involving murine leukemia virus provide indirect evidence that Zn binding is necessary for correct protein function.[13] In these experiments, single and double point mutations, which resulted in replacement of the conserved Cys residues by Ser, afforded mutant viral particles that appeared normal in all respects except that (1) they were noninfectious and (2) they contained cellular RNA instead of viral RNA.[13]

We have prepared Zn^{2+} and Cd^{2+} adducts with the 18-residue peptide comprising the amino acid sequence of the first finger (residues 13 through 30) of NABP p7 from HIV-1 (the causative agent of AIDS). ^1H NMR experiments indicate that the synthetic peptide ($p7^{13-30}$) forms 1:1 metal adducts that are stable in aqueous solution (pH 7, ambient T) for at least a month. Additional Cd^{2+} does not bind to $Cd(p7^{13-30})$ and precipitates from solution as hydroxides at pH 7. Additional Zn^{2+} produces very minor changes in the ^1H NMR spectrum of $Zn(p7^{13-30})$ at pH 7. Except for a few broad ^1H NMR signals observed in the spectrum of the ^{113}Cd adduct (particularly the His-H_2 and -H_4 signals), the ^1H spectra of $Zn(p7^{13-30})$ and $^{113}Cd(p7^{13-30})$ are very similar in appearance. The broader signals of the ^{113}Cd adduct narrow considerably on cooling to -5 °C.

Of the 17 backbone amide protons in $Zn(p7^{13-30})$, 12 protons exhibit resolved multiplets (due to NH-CHα scalar coupling) in the ^1H spectrum obtained at 30 °C (Figure 1). This is in contrast to the broad, unresolved NH signals observed for metal-free $p7^{13-30}$ and reflects the formation of a single, highly stable tertiary structure upon coordination of Zn^{2+}. Many NH ^1H NMR signals

* Author to whom correspondence should be addressed.

(1) Berg, J. M. *Science* **1986**, *232*, 485.
(2) Henderson, L. E.; Copeland, T. D.; Sowder, R. C.; Smythers, G. W.; Oroszlan, S. *J. Biochem.* **1981**, *256*, 8400.
(3) Copeland, T. D.; Morgan, M. A.; Oroszlan, S. *Virology* **1984**, *133*, 137, and references therein.
(4) Karpel, R. L.; Henderson, L. E.; Oroszlan, S. *J. Biol. Chem.* **1987**, *262*, 4961.
(5) Miller, J.; McLachlan, A. D.; Klug, A. *EMBO J.* **1985**, *4*, 1609.
(6) Diakun, G. P.; Fairall, L.; Klug, A. *Nature* **1986**, *324*, 698.
(7) Giedroc, D. P.; Keating, K. M.; Williams, K. R.; Konigsberg, W. H.; Coleman, J. E. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 8452.
(8) Wingender, E.; Seifart, K. H. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 218.
(9) Frankel, A. D.; Berg, J. M.; Pabo, C. O. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 4841.
(10) Parraga, G.; Horvath, S. J.; Eisen, A.; Taylor, W. E.; Hood, L.; Young, E. T.; Klevit, R. E. *Science* **1988**, *241*, 1489.
(11) Smith, L. M.; Jentoft, J. E. *Biophys. J.* **1988**, *53*, 295a; Jentoft, J. E.; Smith, L. M.; Fu, X.; Johnson, M.; Leis, J. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7094.
(12) Schiff, L. A.; Nibert, M. L.; Fields, B. N. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4195.
(13) Gorelick, R. J.; Henderson, L. E; Hanser, J. P.; Rein, A. *Proc. Natl. Acad. Sci.* **1988**, *85*, 8420.

0002-7863/89/1511-0395$01.50/0

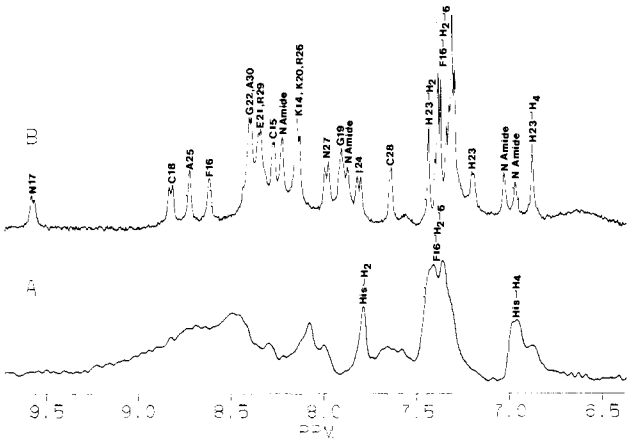


Figure 1. 500-MHz ^1H NMR spectra of the aromatic and amide protons in $p7^{13-30}$ (A: 1.0 mM, T = 28 °C) and $Zn(p7^{13-30})$ (B: 25 mM, T = 30 °C). Both solutions contained 90% H_2O/10% D_2O, pH = 7.0. Signal assignments were made with 2D NMR methods.[22] Data were acquired with a GE GN-500 NMR spectrometer using the 1-3-3-1 pulse sequence[23] (A) and a modified version of the spin–echo pulse sequence[24] (B) and then processed on a VAX computer (FTNMR, Hare Research, Inc.) using 3 Hz (A) and 0.6 Hz (B) exponential line broadening. The peptide was supplied in purified form from Peptide Technologies Corporation (Washington D.C.).

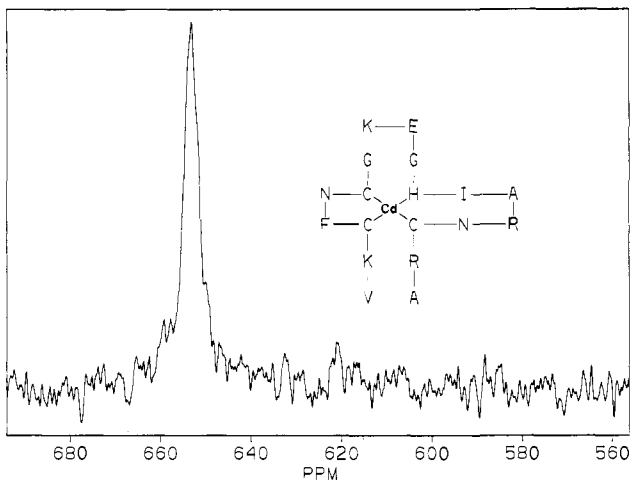


Figure 2. ^{113}Cd NMR spectrum of $Cd(p7^{13-30})$ (20 mM, D_2O, pH* = 7.0, T = 28 °C, Cd^{2+} added as the perchlorate salt, external reference = 0.1 M cadmium perchlorate). Data were acquired on a GE GN-500 NMR spectrometer (110.9 MHz) and processed on a VAX computer (see Figure 1). The amino acid sequence[25,26] and metal binding mode for $Cd(p7^{13-30})$ are shown in the insert.

remain multiplets at temperature as high as 50 °C, providing further evidence for the structural stability of $Zn(p7^{13-30})$.

^{113}Cd NMR spectroscopy provides an attractive and tested means to address metal binding modes in Cd-substituted metalloproteins that normally contain Zn.[14] The ^{113}Cd NMR spectrum of $^{113}Cd(p7^{13-30})$ exhibits a single resonance at δ 653 ppm (Figure 2), which is within the range expected for Cd bound predominantly by S^- donor ligands.[14] For example, the ^{113}Cd NMR spectrum for Cd bound by 4Cys ligands in the structural site of liver alcohol dehydrogenase exhibits a signal at δ 750 ppm,[15] whereas signals for Cd bound by 1Cys, 2His, 1Met[16] ligands in Cd-substituted blue copper proteins are in the range δ 372–432 ppm.[17] Most significantly, the ^{113}Cd NMR spectrum obtained

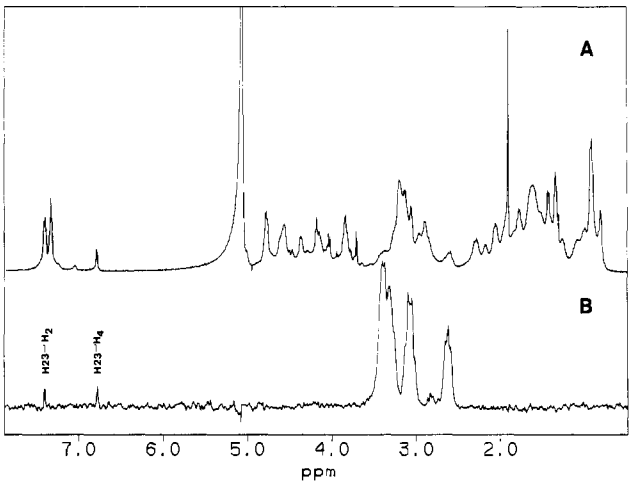


Figure 3. 500-MHz ^1H (A) and ^1H–^{113}Cd heteronuclear spin–echo difference (HSED) (B) spectra of $^{113}Cd(p7^{13-30})$; T = −5.0 °C. The HSED spectrum was obtained by using an 8 ms delay period (τ) with pulse sequence $90_x(H)$–τ–$90_x(Cd)$,$180_y(H)$,$90_{\pm x}(Cd)$–τ–$Acq_{\pm x}$.[19,20]

recently for Cd-substituted gene 32 protein (g32p) from bacteriophage T4 exhibits a signal at δ 638 ppm.[18] g32p binds metals via 3Cys and 1His ligands in the region $-Cys^{77}$-X_3-His-X_5-Cys-X_2-Cys^{90}–.[7,8] The fact that the shift observed for $Cd(p7^{13-30})$ is almost identical with the shift observed for Cd(g32p) ($\Delta\delta$ = 15 ppm) provides strong evidence that Cd is bound by the same heteroatoms (amino acid residues) in these systems.

To determine more precisely the identities of the coordinated ligands, ^1H–^{113}Cd heteronuclear spin–echo difference (HSED) NMR spectroscopy was employed.[19,20] In ^1H–^{113}Cd HSED spectra, only the ^1H signals of protons that are scalar coupled to ^{113}Cd are observed. The ^1H–^{113}Cd HSED spectrum of $^{113}Cd(p7^{13-30})$ (Figure 3B) exhibits signals for both the His-H(2) and -H(4) protons, providing unambiguous evidence that the His imidazole is coordinated to ^{113}Cd via N(3). HSED correlation signals also observed for Cys(β) protons (2.5–3.5 ppm, presently unassigned) confirm the presence of Cd–Cys bonds.

Cross-peak patterns observed recently in the 2D nuclear Overhauser effect spectrum of a synthetic finger peptide from ADR1[10] support the proposal[21] for three-turn α-helical regions in fingers from DNA-binding proteins. Such conformations are precluded by the short peptide sequences of retroviral fingers. Detailed structural analysis of $Zn(p7^{13-30})$ utilizing modern two-dimensional NMR spectroscopy in concert with distance geometry computational methods (underway) will address molecular level aspects of retroviral gene recognition and provide for important comparisons of the structural features of Zn fingers from DNA- and RNA-binding proteins.

Acknowledgment. This work was supported by ACS Institutional Research Grant No. IN-147F, MD Cancer Program/University of Maryland, and by the NOVA Pharmaceutical Corporation. Technical support from Jack Suess (UMBC) and helpful discussions with Drs. L. E. Henderson (Frederick Cancer Research Facility) and R. L. Karpel (UMBC) are gratefully acknowledged.

(14) Summers, M. F. *Coord. Chem. Revs.* **1988**, *86*, 43 and references therein.
(15) Bobsein, B. R.; Myers, R. J. *J. Biol. Chem.* **1981**, *256*, 5313.
(16) ^1H–^{113}Cd NMR correlation experiments indicate that Met may not be bound to Cd in some of the blue copper proteins; see ref 14.
(17) Engeseth, H. R.; McMillin, D. R.; Otvos, J. D. *J. Biol. Chem.* **1984**, *259*, 4822.
(18) Gledroc, D.; Coleman, J. E. In preparation. See also ref 14.
(19) Freeman, R.; Mareci, T. H.; Morris, G. A. *J. Magn. Reson.* **1981**, *42*, 341.
(20) Cohen, J. S.; Chen, C.-W.; Bax, A. *J. Magn. Reson.* **1984**, *59*, 181.
(21) Berg, J. M. *Proc. Natl. Acad. Sci.* **1988**, *85*, 99.
(22) Manuscript in preparation.
(23) Hore, P. J. *J. Magn. Reson.* **1983**, *54*, 539.
(24) Sklenar, V.; Bax, A. *J. Magn. Reson.* **1987**, *74*, 469.
(25) Wain-Hobson, S.; Sonigo, P.; Danos, O.; Alizon, M. *Cell* **1985**, *40*, 9.
(26) *RNA Tumor Viruses, Molecular Biology of Tumor Viruses*, 2nd ed.; Weiss, R., Teich, N., Varmus, H.; Coffin, J., Eds.; Cold Spring Harbor Laboratory, 1985.