A summary of hydrogen-bonded contacts observed in M(allo)$_2$SO$_4$·4H$_2$O complexes is given in Table VIII.

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Registry No. $Co^{II}(allo)_2SO_4·4H_2O$, 115512-16-8; $Ni^{II}(allo)_2SO_4·4H_2O$, 115512-17-9; $Zn^{II}(allo)_2SO_4·4H_2O$, 115512-18-0; $Cd^{II}(allo)_2SO_4·4H_2O$, 115512-19-1.

Supplementary Material Available: Tables of anisotropic thermal parameters and of bonding distances and angles involving H atoms (3 pages); listings of observed and calculated structure factors (74 pages). Ordering information is given on any current masthead page.

Contribution from Immunopharmaceuticals R&D, NTR, Medical Products Department, E. I. du Pont de Nemours & Company, Inc., 331 Treble Cove Road, North Billerica, Massachusetts 01862

Technetium Metallothioneins

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Transchelation of ^{99m}Tc from $TcO(GH)_2$ (GH = glucoheptonate) for Zn^{2+} in metallothionein (MT) was evaluated as a function of MT concentration, pH, buffer, and reaction time. Incorporation of >90% of the ^{99m}Tc presented was achieved in <1 h at [MT] = 7×10^{-6} M in 0.01 M sodium phosphate, pH 6.5. To further understand the chemical nature of the $^{99m}TcMT$ binding, the long-lived ^{99}Tc radionuclide was utilized to prepare macroscopic quantities of TcMT for conventional chemical and spectroscopic analysis. Various $^{99}Tc_x$,ZnMT species were prepared by exchange labeling with $^{99}TcO(GH)_2$ in 0.01 M sodium phosphate, pH 7.0. The incorporation of Tc into Zn_7MT is almost quantitative at low ^{99}Tc/MT ratios. As many as seven Tc atoms could be incorporated into MT. The UV-visible spectra of $^{99}Tc_x$,ZnMT species showed an absorbance at 405 nm with an extinction coefficient of 2500 per Tc atom, which is characteristic with a Tc-thiolate charge-transfer transition. The Raman spectra show a band in the 940-960-cm^{-1} region, indicating that the TcO^{3+} core is bound in square-base-pyramidal geometry. Consumption of MT sulfhydryl groups indicates that initially ^{99}Tc atoms bind with the stoichiometry $^{99}TcO(Cys)_4$. As more ^{99}Tc atoms (three to five) are bound, ligation of additional sulfhydryl groups is not observed, suggesting that Cys residues are bridging TcO^{3+} cores as in $[(^{99}TcO)_2(SCH_2CH_2S)_3]$. Further consumption of Cys residues occurs abruptly with incorporation of the final ^{99}Tc atoms and suggests that the tertiary structure of the MT cluster system has been disrupted.

Introduction

Metallothioneins (MT's) are a class of small, sulfur-rich metal-binding proteins that play an important role in sequestering metal ions in heavy-metal poisoning[1] and probably function in the homeostatis of essential metals like zinc and copper.[2,3] The twenty cysteine (Cys) residues in mammalian thioneins (mol wt 6100) are found at invariant positions in the primary sequences and are responsible for metal binding.[4,5] Zinc, cadmium, and copper are tightly bound in metal thiolate clusters by MT in vivo. Many other metal ions are bound by MT with seven atoms of divalent and up to twenty atoms of monovalent metal ions bound per molecule.[6,7] NMR spectroscopy[8] and X-ray crystallography[9] have determined that two cluster systems are formed as 7-mol quantities of divalent cations are bound in mammalian metallothioneins. The α cluster containing four metal cations bound by eleven Cys thiolates in a bicyclo[3.1.3] structure results from the tetrahedral coordination of thiolates around the metal cations; the β cluster consists of nine Cys thiolates bound by three metal cations in a six-membered ring. The positively charged lysine residues have been postulated to stabilize the tertiary structure of the negatively charged clusters of MT.[10,11] Because of the thermodynamic stability of heavy-metal sulfhydryl bonds,[12,13] MT's offer promise as carriers of diagnostic and therapeutic radionuclides in the radiolabeling of biologically important molecules.[14]

Technetium-99m, a 140-keV γ emitter with a half-life of 6 h, is widely used in diagnostic nuclear medicine and is the radionuclide of choice in the radiodiagnosis of diseases. Monoclonal antibodies have been radiolabeled with technetium either by direct means[15,16] or through the use of bifunctional chelating agents including DTPA (diethylenetriaminepentaacetic acid)[17,18] and DADS [*N*,*N*'-bis(mercaptoacetamido)ethylenediamine].[19] Since Tc is known to form inert coordination complexes with aliphatic thiols, we have investigated the use of MT as a bifunctional chelator for the labeling of monoclonal antibodies with technetium. In this paper, we describe the synthesis and characterization of ^{99m}Tc and ^{99}Tc,ZnMT's. With the use of ^{99}Tc, a 85-keV (average) β-emitting isotope with a half-life of 2.12×10^5 years, macroscopic amounts of ^{99}Tc,ZnMT's were prepared and characterized by chemical and spectroscopic methods. The formation of $TcO(Cys)_4$

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units within the ZnMT cluster systems are discussed. Kinetic studies of ^{99m}Tc incorporation into ZnMT by exchange from the ^{99m}TcO(glucoheptonate)$_2$ complex[20], $^{99m}TcO(GH)_2$, are described. The radiolabeling of biologically active molecules, especially monoclonal antibodies, at inherently low concentrations, is demonstrated.

Experimental Section

All reagents were metal-free. Metal content was determined by inductively coupled plasma (ICP) spectrophotometry using a Plasmatherm crystal-controlled radiofrequency generator equipped with a Minuteman 305SMP monochromator. ^{99}Tc was counted in 10 mL of Aquasol (NEN-Du Pont) in glass scintillation vials by using a Packard Model 460 liquid scintillation counter. Formation of $TcO(GH)_2$ complexes and kinetic measurements of the ^{99m}Tc transchelation reaction were monitored by using a conventional radio-TLC scanner[21] equipped with an Eberline RM19 rate meter and a Hewlett-Packard 3390A integrator. UV–visible spectra were taken on a Perkin-Elmer 3840 Lamda diode-array spectrophotometer. Raman spectroscopy was carried out on a Coherent Radiation monochromator equipped with an RCA 31034 photomultiplier tube.

1. Preparation of Rabbit Liver Thionein. MT was induced in rabbits by 15 subcutaneous injections at 2–3-day intervals of 1 mg of $CdCl_2$/kg of body weight. Rabbit MT-1 was isolated by a procedure adapted from Kimura[22] and Kagi.[23] Thionein, the metal-free protein, was generated by dialysis (2000 MWCO, Spectrophor Co.) against 0.1 M HCl and was subsequently purified by size exclusion chromatography according to the method of Good et al.[24] The purity of the material was established by amino acid analysis (Durrum D-500).

2. Preparation of Rabbit Liver ZnMT-1. Thionein was treated with a 100-fold excess of dithiothreitol (DTT) in 25 mM [tris(hydroxymethyl)aminomethane] (Tris), pH 8.0, under anaerobic conditions for 4 h. The DTT was removed by G-25 size exclusion chromatography with 0.02 M HCl as eluent. Thionein concentration was determined by using the thiol-specific reagent 2,2′-dithiodipyridine (Aldrich Chemical Co.).[25] Reconstitution of thionein was accomplished by addition of 14 equiv of $ZnSO_4 \cdot 7H_2O$ (Puratronic, Alfa Chemical Co.) followed by titration to pH 7.0 with 0.5 M Tris. Excess Zn was removed by dialysis against 0.01 M sodium phosphate, pH 7.0, using 2000 MWCO tubing (Spectropor Co.). Final metal stoichiometry was determined to be Zn_7MT.

3. Preparation of the α and β Fragments of Rabbit Liver ZnMT-1. The α and β fragments of ZnMT were generated by proteolytic cleavage with subtilisin according to the methods of Nielson and Winge.[26] Final metal stoichiometries were determined to be α-Zn_4MT and β-Zn_3MT.

4. Preparation of $^{99m}TcO(GH)_2$. $^{99m}TcO_4^-$ was obtained by eluting a $^{99}Mo/^{99m}Tc$ generator (E. I. du Pont de Nemours & Co., Inc.) with 5.8 mL of 0.15 M NaCl. $^{99m}TcO(GH)_2$ was made by reacting 0.5 mL of $Na^{99m}TcO_4$ eluate with stannous glucoheptonate,[27] available in kit form as Glucoscan (E. I. du Pont de Nemours & Co., Inc.). With use of a conventional radio-TLC scanner, the percentages of $^{99m}TcO_4^-$, $^{99m}TcO_2$, and $^{99m}TcO(GH)_2$ in the kit were determined by ITLC™-SG (Gelman Sciences). Elution with saline determined the percent $^{99m}TcO_2$ at the origin with $^{99m}TcO(GH)_2$ and $^{99m}TcO_4^-$ at the solvent front; elution with methyl ethyl ketone determined the percent $^{99m}TcO_2$ and $^{99m}TcO(GH)_2$ at the origin with $^{99m}TcO_4^-$ at the solvent front. Preparations of $^{99m}TcO(GH)_2$ used to label ZnMT species contained less than 3% total ^{99m}Tc as $^{99m}TcO_2$ or $^{99m}TcO_4^-$.

5. Preparation of $^{99}TcO(GH)_2$. $(NH_4)^{99}TcO_4$ (Oak Ridge National Laboratories) was purified by aqua regia oxidation and recrystallization. Stock soltuions were standardized by ICP spectrophotometry and liquid scintillation counting (LSC). Stannous glucoheptonate was prepared by dissolving $SnCl_2$ (Alfa Chemical Co.) in 1.0 M NaOH in the presence of a 20-fold molar excess of GH. $(NH_4)^{99}TcO_4$ solutions were spiked with a tracer amount of $Na^{99m}TcO_4$ to allow determination of $^{99}TcO(GH)_2$ formation. An aliquot of a standardized solution of $(NH_4)^{99}TcO_4$ was added to the stannous GH solution resulting in a Sn/Tc ratio of 1.5. The $^{99}TcO(GH)_2$ complex was then adjusted to pH 6.5 with 0.5 M Na_2HPO_4. The same ITLC™-SG system and criteria used to assess $^{99m}TcO(GH)_2$ formation was applied to determine the integrity of the $^{99}TcO(GH)_2$ preparation.

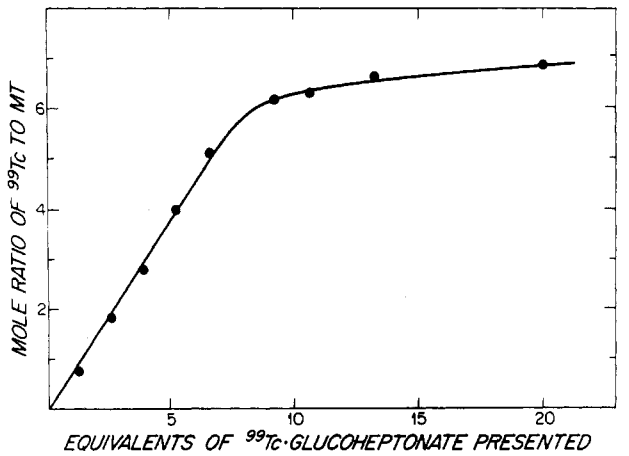

Figure 1. Mole ratio of ^{99}Tc incorporated as a function of $^{99}TcO(GH)_2$ presented in the exchange labeling of ZnMT.

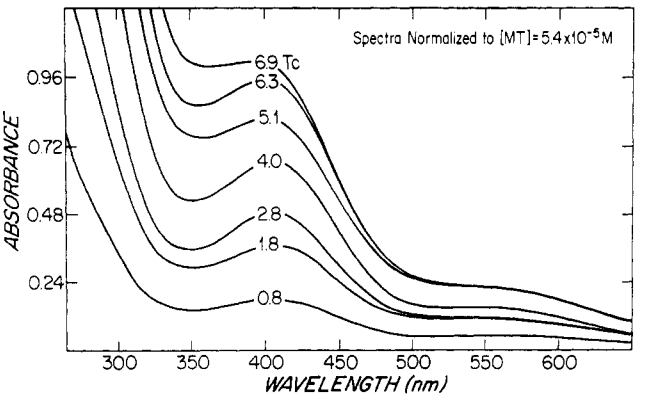

Figure 2. UV–visible spectra of various ^{99}Tc,ZnMT species containing 0.8–6.9 mol of ^{99}Tc/molecule of MT. The spectra are normalized to [MT] = 5.4×10^{-5} M.

6. Preparation of ^{99m}Tc,ZnMT and α- and β-^{99m}Tc,ZnMT Fragments. ^{99m}Tc,ZnMT species was generated by reacting 1 mCi of $^{99m}TcO(GH)_2$ with 1 mL of various concentrations of ZnMT ranging from 10^{-4} to 10^{-7} M in 0.01 M sodium phosphate, pH 6.5. The percent transchelation at 0.5, 1, 4, and 24 h was determined in duplicate by radioactivity analysis of ^{99m}Tc species on ITLC™-SG eluted with saline where ^{99m}Tc,ZnMT remains at the origin.

7. Preparation of ^{99}Tc,ZnMT. $^{99}TcO(GH)_2$ was added to ZnMT to generate reactions resulting in $^{99}TcO(GH)_2$/ZnMT ratios ranging from 1 to 20. The final concentration of ZnMT was 5×10^{-5} M in 50 mM sodium phosphate, pH 7.0. Reactions were stopped after 4 h by dialysis using 2000 MWCO tubing (Spectropor Co.). The ^{99}Tc,ZnMT species were then exhaustively dialyzed against 50 mM Tris-Cl, pH 7.0, until no ^{99}Tc could be detected in the dialysate by LSC.

Results and Discussion

Transmetalation of metal ions commonly bound by mammalian MT is an important process that follows the thermodynamic metal-binding constants,[28] e.g. $Cu^+ \sim Hg^{2+} > Cd^{2+} > Zn^{2+}$. Thus, the treatment of calf liver Cu,ZnMT with Cd^{2+} leads to the selective and complete displacement of Zn^{2+} resulting in a Cu,CdMT species.[29] Complete transmetalation of Hg^{2+} for Zn^{2+} is also observed in reconstituted rabbit MT at neutral pH.[30] $TcO(GH)_2$ prepared with the long-lived nuclide ^{99}Tc was utilized in transchelation reactions to prepare macroscopic amounts of various ^{99}Tc,ZnMT's for characterization by conventional chemical and spectroscopic methods. Incorporation of ^{99}Tc into ZnMT as a function of [$^{99}TcO(GH)_2$] is shown in Figure 1. Each point

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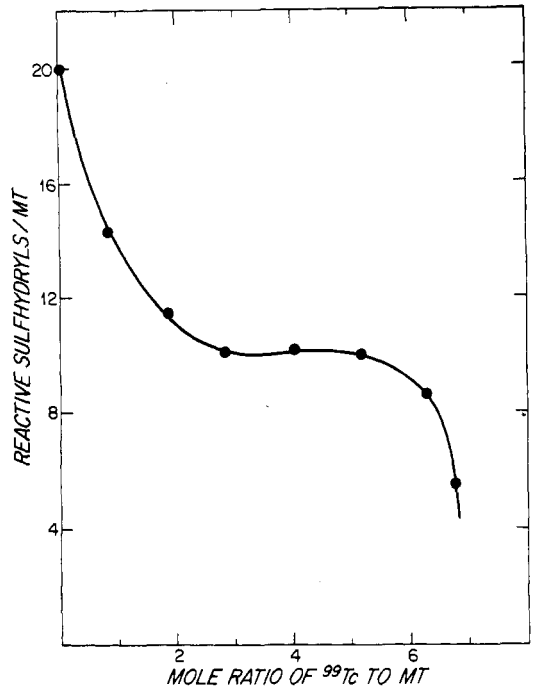

Figure 3. Determination of sulfhydryl groups in ^{99}Tc,ZnMT species as detected by 2,2'-dithiodipyridine at pH 4.0.

on the graph represents the molar equivalents of ^{99}Tc found in the products of individual reactions combining a known quantity of ZnMT with varying molar equivalents of $^{99}TcO(GH)_2$. ^{99}Tc,ZnMT species containing between 0.8 and 6.9 atoms of ^{99}Tc were obtained. The exchange of the first Tc atoms into ZnMT is almost quantitative. As the $^{99}TcO(GH)_2$/ZnMT ratio is increased, the efficiency of transchelation decreases and appears to saturate the binding capacity of MT. The number of Tc atoms incorporated approaches 7 as a large excess of $^{99}TcO(GH)_2$ is presented. The UV–visible spectra of the various ^{99}Tc,ZnMT species prepared are shown in Figure 2. The absorbance at 405 nm increases linearly with ^{99}Tc incorporation. This electronic absorption was determined to have an extinction coefficient of 2500 M^{-1} cm^{-1} per ^{99}Tc equivalent and is characteristic of the Tc–thiolate (Tc ← S) charge-transfer transition previously observed in the oxobis(dithiolato)technetium(V) complexes.[13] The Raman spectra of the ^{99}Tc,ZnMT species described show intense features in the 940–960 cm^{-1} region and indicate the presence of a TcO^{3+} core in square-based-pyramidal geometry.[12] Since the Raman spectra were obtained at a ^{99}Tc,ZnMT concentration of 3.9×10^{-5} M and the exciting wavelength is near λ_{max} for the (Tc ← S) charge-transfer band, an electronic–vibrational resonance is occurring. Thus ^{99}Tc transchelation is efficient and results in ^{99}Tc,ZnMT species with varying ^{99}Tc/Zn stoichiometries characterized by spectral properties corresponding to $[^{99}TcOS_4]^-$.

In native MT, Zn^{2+} ions are bound in tetrahedral geometries by both bridged and nonbridged thiolate ligands. Five of the seven Zn^{2+} ions are bound by two bridging and two nonbridging cysteine thiolates. The remaining two Zn^{2+} ions are bound by one nonbridging and three bridging cysteine thiolates. If the seven TcO^{3+} cores introduced into the MT are bound exclusively by the twenty cysteinyl residues, some thiolate groups must bridge TcO^{3+} cores to supply four ligands for each Tc atom. To show the presence of bridging thiolate ligands in ^{99}Tc,ZnMT, the disappearance of reactive thiolates with increasing ^{99}Tc incorporation was quantitated by using the sulfhydryl specific reagent 2,2'-dithiodipyridine at pH 4.0 (see Figure 3). At pH 4.0, Zn^{2+} ions are no longer bound by sulfhydryl groups, while the $^{99}TcOS_4^-$ complex remains intact. The data indicate that initially $^{99}TcO^{3+}$ cores are bound with the stoichiometry $TcO(Cys)_4^-$. However, binding of further ^{99}Tc atoms (Tc/MT = 3–5) occurs without consumption of additional thiolates and must be attained through sharing of cysteine thiolates and/or through coordinating of amine or amide nitrogen

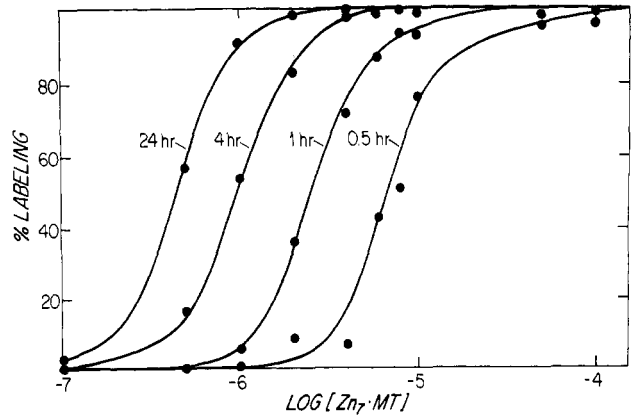

Figure 4. Incorporation of presented ^{99m}Tc as a function of MT concentration in the exchange labeling of ZnMT by $^{99m}TcO(GH)_2$ in 0.01 M phosphate buffer (pH 6.5).

atoms. Since the intensity of the Tc ← S charge-transfer absorption continues to increase through this region, the TcO^{3+} cores are most likely bridged by sulfhydryl groups in a manner similar to that observed in the bridged dimer $[(TcO)_2(SCH_2CH_2S)_3]$.[31,32] Further consumption of thiolate groups occurs abruptly with incorporation of the final ^{99}Tc atoms and suggests that the tertiary structure of the MT cluster system has been disrupted. Significant changes in the tertiary structure of the tetrahedral-based ZnS_4 cluster systems would be expected upon replacement of tetrahedral Zn^{2+} by TcO^{3+} cores with square-base-pyramidal coordination. Additional characterization of the various ^{99}Tc,ZnMT species is required to demonstrate the presence of Tc–S–Tc bridging and to elucidate the changes in tertiary structure of native MT that accompany Tc incorporation.

The use of the short-lived ^{99m}Tc in diagnostic nuclear medicine requires that radiolabeling occur immediately before use. Therefore, for mammalian ZnMT to function as an effective carrier of ^{99m}Tc in the radiolabeling of biologically active molecules, transchelation of Tc for Zn must be rapid and efficient, i.e. >90% incorporation within 1 h. pH studies performed to maximize transchelation efficiency showed that Zn^{2+} ions are labile at pH ≤7 in phosphate buffer, and facile exchange with the TcO^{3+} core occurs. The percent transchelation of $^{99m}TcO^{3+}$ at pH 6.5 at reaction times of 0.5, 1, 4, and 24 h as a function of ZnMT concentration is shown in Figure 4. The curves indicate rapid and efficient exchange at MT concentrations $>10^{-5}$ M. At MT concentrations below 10^{-5} M, however, this efficiency decreases dramatically, and incorporation of >90% of the ^{99m}Tc presented could be acheived only after reaction times greater than 1 h. Comparable kinetics were observed with the α-ZnMT and β-ZnMT fragments. Since the concentration of "carrier-free" $^{99m}Tc(GH)_2$ is only 10^{-8} M, kinetics of the labeling reaction are strongly affected by diffusion at low ZnMT concentrations. Although the transchelation reaction would be expected to be a multistep phenomenon, radiolabeling of ZnMT appears to be characterized by simple second-order kinetics limited by diffusion of the reactants. Similar kinetics have also been observed to occur with other "carrier-free"-radionuclide/chelator reactions (e.g. ^{111}In/DPTA) at comparable chelator concentrations.[33] Hence, the $^{99m}TcO^{3+}$ core can be transchelated from the GH complex to ZnMT, α-ZnMT, and β-ZnMT with high efficiency to form thermodynamically stable ^{99m}Tc,ZnMT species.

Conclusion

Metallothioneins offer promise as a carrier of technetium in radiolabeling of biologically active molecules. Metallothionein binds technetium(V) in a thermodynamically stable and kinetically

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inert thiolate complex that is stable to oxidation and transchelation in vivo.[34] As demonstrated by UV-visible and Raman spectroscopy, the TcO^{3+} core is bound in square-based-pyramidal geometry to give $TcOS_4^-$ stoichiometry. As many as seven Tc atoms were observed to exchange into ZnMT, and Tc-S-Tc bridging is postulated to accommodate the $TcOS_4^-$ stoichiometry.

Metallothionein has functional groups appropriate for its conjugation to biologically important molecules. MT's conjugated to monoclonal antibodies maintain their immunoreactivity.[34] The kinetics of exchange of Tc(V) into MT are rapid and will allow efficient radiolabeling of MT-antibody conjugates with ^{99m}Tc immediately before use. The application of these ^{99m}TcMT-labeled MAb's in diagnostic nuclear medicine is ongoing.

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Registry No. Cys, 52-90-4.

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Electron Transfer. 94. Internal Redox in Cobalt(III)-Bound Hypophosphite[1]

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The hypophosphito derivative of Co^{III}, $(NH_3)_5CoO_2PH_2^{2+}$, decomposes in basic media, yielding Co(II) quantitatively along with a 1:1 mixture of hypophosphite and phosphite. When this reaction is carried out in 0.014-0.60 M OH^- in the presence of Na_4EDTA, a strongly absorbing intermediate ($\epsilon_{295} = 1.1 \times 10^3$ M^{-1} cm^{-1}) is formed and then undergoes decay. Kinetic profiles are consistent with a pair of consecutive pseudo-first-order processes, each of which is also very nearly first order in OH^-. Isotopic labeling experiments with the $-PD_2$ analogue of the complex indicate a kinetic isotope effect, k_{PH_2}/k_{PD_2}, of 4.0 ± 0.5 for the first step in the sequence and 2.0 ± 0.2 for the second. Our results suggest that the reaction is initiated by removal of a P-bound proton from the hypophosphite ligand, forming conjugate base I. The latter then reacts with a second OH^- and undergoes a hydride shift from phosphorus to cobalt(III), generating a cobalt(I) species, which rapidly reduces an additional molecule of the Co(III) reactant to Co(II). If the reaction is carried out in the presence of a second cobalt(III) oxidant, the latter competes with the hypophosphito complex for the Co(I) intermediate, lowering the yield of free $H_2PO_2^-$. From the ratio of phosphite to hypophosphite, the relative reactivities of the external "trap" and the hypophosphito complex toward Co(I) may be estimated. The formato complex of $(NH_3)_5Co^{III}$ reacts over twice as rapidly as the analogous pyridine complex, a reversal of the selectivity observed in outer-sphere reduction series, thus implying the use of an inner-sphere path in the Co(I)-formato reaction. The complex $(NH_3)_5Co(NCS)^{2+}$ is an especially efficient trap for Co(I), reflecting the unusually soft character of the Co(I) center. The same transformation of the hypophosphito complex, when carried out at pH 8.7-11 in the absence of added NaOH, generates a straightforward exponential profile without indication of an intermediate and exhibits a kinetic isotope effect of only 2.1-2.3, indicating that the predominant mechanism under these conditions is different from that operating in the more strongly basic systems here examined.

The hypophosphito derivative of $(NH_3)_5Co^{III}$ may be prepared and stored without difficulty.[2,3] When treated with basic species in aqueous media, it undergoes an internal redox process, yielding Co(II) and equimolar quantities of free phosphite and hypophosphite (eq 1). It was proposed[3] that this transformation, which

$$2[(NH_3)_5Co(OPH_2O)]^{2+} + H_2O \xrightarrow{B:} 2Co^{II} + HPO_3^{2-} + H_2PO_2^- + 3NH_4^+ + 7NH_3 \quad (1)$$

does not proceed with uncoordinated hypophosphite or with bound or unbound phosphite, is initiated by a hydroxide-induced hydride shift from P(I) to Co(III), yielding a Co(I) intermediate, which then rapidly reduces unreacted Co(III) to Co(II).

The reaction appears to exhibit a straightforward kinetic picture at pH 9-11 but becomes complex in more highly basic solutions, passing through a strongly absorbing intermediate, although the net stoichiometry is unchanged. The present report deals with this reaction under the latter conditions. In addition to our attempt to address this complexity, we report experiments designed to bear upon the selectivity of the Co(I) intermediate toward several Co(III) oxidants.

Experimental Section

Physical Methods. ^1H and ^{31}P NMR spectra were recorded on a GN300 spectrometer using DSS and 85% external H_3PO_4 as chemical shift standards.[4] Infrared spectrometry was performed on a Perkin-Elmer 283 instrument. Kinetic measurements and UV-vis spectra were obtained on either a Beckman UV 5260 or Perkin-Elmer Lambda 4B spectrophotometer.

Materials. The $(NH_3)_5Co^{III}$ complex of hypophosphorous acid, $[(NH_3)_5CoO_2PH_2][ClO_4]_2$, and its $-PD_2$ analogue were prepared as described;[3] chromatographic purification of the deuteriated complex was carried out in D_2O rather than in H_2O. The deuterio derivative was found to contain 6% $[CoO_2PHD]^{2+}$ by ^{31}P NMR.[5] Additional (N-$H_3)_5Co^{III}$ derivatives of H_2O,[6] formate,[7] pyridine,[8] and thiocyanate (N-bound)[9] were prepared by literature methods. Lithium perchlorate[10] was doubly recrystallized and Na_4EDTA (Aldrich) recrystallized once before use. Stock solutions of carbonate-free NaOH were standardized against potassium hydrogen phthalate (primary standard grade).

General Considerations. All solutions were rigorously deaerated prior to use, and all transfers were done by syringe under N_2. Determination of phosphite and hypophosphite in solutions containing both states utilized the iodometric method of Jones and Swift.[11]

Labeling Studies. Reaction of the hypophosphito complex (0.0125 M) with acetate buffer (0.015 M, pH 4) in D_2O was monitored by ^1H NMR.

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(4) "DSS" = $(CH_3)_3Si(CH_2)_2SO_3^-Na^+$; upfield singlet at $\delta = 0$. ^1H spectra were recorded with frequency 300.521 MHz, spectral width 4 kZh, pulse width 5 μs, and repetition time 5.3 s. Respective parameters for ^{31}P spectra were 121.652 MHz, 16 kHz, 3 μs, and 9.1 s. Each spectrum was generated from 32768 data points.
(5) Infrared spectrometry (Nujol): ν_{P-H} 2397 cm^{-1}; ν_{P-D} 1710 cm^{-1}. The NMR characterization of these complexes has been described.[3]
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