J. Am. Chem. Soc. **1984**, *106*, 2812-2818

Nuclear Magnetic Resonance, Crystallographic, and Hydrolytic Studies of the β- and γ-Coordination Isomers of Pentaammine(dihydrogen triphosphato)cobalt(III) Monohydrate

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Abstract: The linkage isomers β-(dihydrogen triphosphato)pentaamminecobalt(III) and γ-(dihydrogen triphosphato)pentaamminecobalt(III), $[Co(NH_3)_5H_2P_3O_{10}\cdot H_2O]$, have been synthesized and characterized by ^{31}P NMR spectroscopy and by X-ray crystallography. The crystal structures were solved by the heavy-atom technique and refined to *R* indices of 0.036 and 0.042 for the β and γ isomers, respectively. The average Co–O coordination distance for the two structures is 1.92 Å while the average Co–N coordination distance is 1.95 Å. The β-monodentate structure exhibits two intramolecular hydrogen bonds between the pentaamminecobalt moiety and the triphosphate chain; no intramolecular hydrogen bonds are observed for the γ-monodentate isomer. The phosphate hydrolysis rates of the isomers have been studied in the presence of *cis*-$[Co(cyclen)(OH)_2]^+$, where cyclen is 1,4,7,10-tetraazacyclotetradecane. At pH 9.0 and 40 °C, β-$[Co(NH_3)_5P_3O_{10}]^{2-}$ hydrolyzes over 50 times faster than the corresponding γ isomer. In addition, the amounts of acid consumed upon mixing solutions of the isomers with solutions of the cyclen complex differ sharply. A mechanism including competition between chelation and hydrolysis is offered to explain the observed differences in hydrolysis rates and in hydrogen ion consumption.

The enzyme-catalyzed hydrolysis of ATP accompanied by the liberation of energy plays a key role in the energy metabolism of biological systems. Enzymes which catalyze phosphate hydrolysis typically require one or more metal ions for activity.[1] Magnesium(II) is one of the most common metal ion activators in vivo. It can enhance phosphate hydrolysis via charge neutralization by coordination to one, two, or three phosphate oxygen atoms of the nucleotide, forming mono-, bi-, or tridentate coordination complexes, respectively. Unfortunately, the various coordination modes of the magnesium(II)–triphosphato complexes rapidly interchange in solution, thereby preventing their separation and characterization. Certain complexes of cobalt(III) have been found to have large catalytic effects on phosphate hydrolysis reactions.[2-5] In contrast to the Mg(II) complexes, the corresponding Co(III) coordination complexes are sufficiently stable to permit isolation, crystallization, and examination of the diamagnetic cobalt(III)–phosphato complexes by ^{31}P NMR spectroscopy. These cobalt complexes have been employed to study variations in enzyme reactivity toward substrates with differing coordination modes.[6,7]

The crystal structures of the following Co(III) bidentate and tridentate chelation complexes with triphosphate have already been reported: β,γ-bidentate $Co(NH_3)_4H_2P_3O_{10}\cdot H_2O$,[8] α,γ-bidentate $Co(NH_3)_4H_2P_3O_{10}\cdot H_2O$,[9] and α,β,γ-tridentate $Co(NH_3)_3H_2P_3O_{10}$.[10] The phosphate hydrolysis of $Co(NH_3)_4H_2P_3O_{10}$ has been investigated in detail.[4,11-13] For the present study, the β- and γ-monodentate isomers of $Co(NH_3)_5H_2P_3O_{10}$ (the α- and γ-monodentate isomers being equivalent in the absence of nucleotide) have been synthesized and characterized by both ^{31}P NMR and X-ray crystallography. Hydrolysis rate studies in the presence and absence of *cis*-$[Co(cyclen)(OH)_2]^+$ have been conducted. Observed differences in rates of hydrolysis contribute to the understanding of phosphate hydrolysis reactions catalyzed by metal ions and have implications for the mode of coordination of Mg(II) to ATP at the enzyme active sites.

Experimental Section

General. Reagent grade chemicals were used throughout. Cation and anion resins were Bio-Rad analytical grade, 100–200 mesh, AG-50W-X2 and AG-1-X2. The method of Basolo and Murmann[14] was used to prepare $[Co(NH_3)_5CO_3]NO_3\cdot H_2O$. Tetramethylammonium chloride was purified by filtration and recrystallization from water followed by washing of the crystals with ethanol and chloroform.[15]

The ligand 1,4,7,14-tetraazacyclotetradecane (cyclen) was prepared as described in the literature[16] and the *cis*-$[Co(cyclen)Cl_2]Cl$ complex was prepared by the method of Collman and Scheider.[17]

Synthesis of β- and γ-$Co(NH_3)_5H_2P_3O_{10}\cdot H_2O$. A 1.10-g sample of $[Co(NH_3)_5CO_3]NO_3\cdot H_2O$ was dissolved in 200 mL of water. The pH was lowered to 2.5 by addition of 1.0 M nitric acid to cause decarboxylation and yield a solution 0.200 M in $[Co(NH_3)_5H_2O]^{3+}$. An equal volume of 0.022 M $Na_5P_3O_{10}$ solution was adjusted to pH 4 with 1.0 M HNO_3 and was slowly added to the first solution. The pH was maintained at 3 by addition of 1.0 M HNO_3 to prevent the turbidity which appears at higher pH. The slight molar excess of $Na_5P_3O_{10}$ gave the maximal yield; a much greater excess or more highly concentrated solutions resulted in the formation of crystals shown to be the triphosphate salt of aquopentaamminecobalt(III). The reaction solution, 0.010 M in $[Co(NH_3)_5H_2O]^{3+}$ and 0.011 M in $Na_5P_3O_{10}$, was heated for 10 min at 80 °C in a water bath. During heating, the color changes from red-orange to deep red.

The reaction solution was cooled, filtered to remove any solid impurities, and passed through a 10 mm × 200 mm cation-exchange column, Na^+ form. The β- and γ-$Co(NH_3)_5H_2P_3O_{10}$ isomers moved rapidly through the column when eluted with water. Pentaammine(pyrophosphato)cobalt(III), formed as a reaction byproduct, remained tightly bound to the cation column along with other reaction byproducts. The

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0002-7863/84/1506-2812$01.50/0

Table I. Crystallographic Parameters for the β- and γ-Monodentate Isomers of Pentaammine(dihydrogen triphosphato)cobalt(III) Monohydrate

isomer	β-$Co(NH_3)_5H_2P_3O_{10}$	γ-$Co(NH_3)_5H_2P_3O_{10}$
space group	$P2_12_12_1$	$P2_12_12_1$
cell constants		
a, Å	7.198 (1)	7.759 (1)
b, Å	13.735 (1)	13.394 (2)
c, Å	14.112 (2)	13.488 (2)
V, Å^3	1395.2 (5)	1401.7 (5)
Z	4	4
calcd density, g cm^{-3}	1.985	1.976
absorption coeff, mm^{-1}	13.8	13.7
total reflections	1219	1222
intensities > $2\sigma(I)$ (used for structure analysis)	1097	1174
final *R* index	0.036	0.042

pyrophosphato species was recovered in a 5% yield upon elution with 0.3 M aniline as described in the literature.[3]

The pH of the solution containing the β- and γ-triphosphate isomers was raised to 7.6 with 1.0 M KOH and charged onto a 25 mm × 280 mm anion-exchange column, Cl^- form. The isomers formed a tightly bound red band at the top of the column. The column was washed with two column volumes of water and a 0.05 M formic acid solution adjusted to pH 4 with 1.0 M KOH. A red solution containing the β-isomer eluted off the column in about 4 h while the γ-isomer, which remained bound to the column, was subsequently eluted with 0.05 M HCl.

The solution containing the β-isomer was raised to pH 7.6 with 1.0 M KOH and charged onto a 10 mm × 140 mm anion-exchange column, Cl^- form. The column was washed with two column volumes of water, and the β-isomer was eluted with 0.05 M HCl.

Ethanol was added dropwise with stirring to both solutions containing the individual isomers until cloudiness occurred and lasted for about 2 s before disappearing. The solutions were stored at 0 °C. Long needle-shaped crystals of the γ-isomer grew overnight, and smaller crystals of the β-isomer grew in 2–4 days. The crystals were filtered, washed with ethanol and diethyl ether, and dried overnight in a vacuum desiccator over silica gel.

Anal. Calcd for $Co(NH_3)_5H_2P_3O_{10}\cdot H_2O$: H, 4.59; N, 16.79; Co, 14.13; P, 22.28. Found for the β-isomer: H, 4.61; N, 16.74; Co, 14.29; P, 22.36; yield, 13.5%. Found for the γ-isomer: H, 4.43; N, 16.63; Co, 14.01; P, 22.60; yield, 10.5%.

NMR. ^{31}P NMR spectra were recorded on a 90-MHz JEOL FS90Q instrument using 50% H_3PO_4 as an external standard. The sample solutions contained 33% D_2O to provide a signal lock and were 0.1 M in disodium ethylenediamminetetraacetate to prevent line broadening by any paramagnetic cobalt(III) species that may be present. The pH was adjusted to 8.0 with 1.0 M NaOH and measurements were recorded at ambient temperature.

Visible Spectra. Visible spectra were recorded with a Cary 219 spectrophotometer. Crystals of the complexes were dissolved in 0.1 M $NaHCO_3$ and the pH was brought to 8.0 with 0.1 M NaOH. The β-isomer has maxima at 519 (ϵ 74 M^{-1} cm^{-1}) and 358 nm ($\epsilon = 59$) while the γ-isomer has maxima at 520 (ϵ 61 M^{-1} cm^{-1}) and 355 nm ($\epsilon = 53$).

Crystal Structure Analyses. X-ray intensity data for both crystals were collected up to a 2θ limit of 120° on an Enraf-Nonius CAD4 diffractometer using Cu Kα radiation ($\lambda = 1.5418$ Å). Crystallographic parameters are presented in Table I. The data were subjected to the usual corrections for crystal decay, Lorentz, polarization, and absorption effects.

The cobalt position for both structures was located from Patterson maps. Subsequent structure factor and Fourier calculations using the phases derived from the cobalt positions revealed all remaining non-hydrogen atoms. These atoms were refined by the full-matrix least-squares technique using anisotropic temperature factors and a weighting scheme based on counting statistics with the weight of each reflection proportional to $1/[\sigma^2(F) + (pF_o)^2]$ where $p = 0.02$ for the β-isomer and $p = 0.03$ for the γ-isomer. The *R* indices at this point ($\sum||F_o| - |F_c||/\sum|F_o|$) were 0.046 and 0.049, for the β- and γ-forms, respectively.

Since both crystals are in a noncentrosymmetric space group, they contain only one of two possible conformational enantiomorphs. The correct enantiomorphs in the crystals were determined by refining the enantiomeric structure with all coordinates inverted. This resulted in *R* indices of 0.127 and 0.130, indicating that the original coordinates were correct. The rather large differences of about 0.08 in the *R* indices of the enantiomorphs arise from the fact that Co is near the absorption edge for Cu radiation, resulting in a large anomalous effect. This effect becomes more noticeable for reflections at higher θ angles where the contribution of anomalous scattering to the total reflection intensity increases since the nonanomalous scattering decreases significantly for all the atoms.

At this point hydrogen atoms were located on difference Fourier maps. The non-hydrogen atoms were further refined with the contribution of the hydrogen atoms included in the structure factors. The positions of all the hydrogen atoms except H(O1P3) for the β-monodentate form were subsequently refined by using a fixed temperature factor of 4.0 Å^2. H(O1P3) was fixed on a vector between O1(P3) and the hydrogen bond acceptor O(W2). The final *R* indices were 0.036 and 0.042 for the β- and γ-monodentate forms, respectively. At the conclusion of refinement, the maximum shift/error ratio was 0.01 for non-hydrogen atoms in both structures.

Acid Ionization Constants. The isomers were titrated with 0.100 M KOH in a water-jacketed cell. The ionic strength was maintained at 1.0 with tetramethylammonium chloride. The potassium hydroxide solution was delivered in microliter increments from a glass syringe driven by a step motor controlled by an Apple II computer.[18] The pH was measured with an Orion Model 701-A pH meter using a combination electrode.

Kinetics. Hydrolysis of the compounds was followed by measuring the production of orthophosphate ion by a spectrophotometric phosphomolybdate method.[4,19] Tetramethylammonium chloride was used to maintain an ionic strength of 1.0 since this salt does not have the usual catalytic effect on hydrolysis[20] observed with metal cations.

In a typical experiment, 0.50 mL of a solution that was 40 mM *cis*-$[Co(cyclen)Cl_2]Cl$ and 1.0 M tetramethylammonium chloride was adjusted to pH 9.0 by the addition of 1.0 M KOH and incubated at 40 °C for 30 min to generate *cis*-$[Co(cyclen)(OH)_2]^+$.[21] The pH of this solution was then adjusted to the desired reaction pH. An equal volume of solution 50 mM in the cobalt–triphosphate species and 1.0 M in tetramethylammonium chloride was prepared and adjusted to the desired reaction pH with 1.0 M KOH.

The two solutions were combined and the pH was maintained by the addition of either 1.0 M HNO_3 or 1.0 M KOH with use of a pH stat which was controlled by an Apple II microcomputer[18] interfaced with an Orion Model 701-A pH meter. The amounts of acid and base consumed were plotted and stored by the Apple II computer.

To prevent the introduction of carbonic acid and to reduce volume changes due to evaporation, the reaction was run in an atmosphere of nitrogen gas that had been passed through a soda lime tube and bubbled through water.

At appropriate time intervals, 50–100-μL samples were taken and quenched in 700 μL of cold water with a calculated amount of 1.0 M HNO_3 to bring the reaction to pH 5. (At this pH the hydrolysis process has its slowest rate.[13]) These samples were treated with 200 μL of 0.2 M vanadium(II) freshly prepared by passing 0.1 M V_2O_5 in 2 M HCl through a Jones reductor. Each sample was allowed to stand with the vanadium(II) for 2 min to fully reduce the cobalt(III) to cobalt(II) and release all bound phosphates. Since vanadium(II) interferes with phosphate analysis, it was removed by passing the sample solution through a 1.0-mL column of cation-exchange resin. The column was washed with 1-, 2-, and 1-mL volumes of water and the volume of the collected solution was brought to 5.0 mL. A reagent for phosphate analysis[19] was freshly prepared with 1 volume of 6 N H_2SO_4, 2 volumes of water, 1 volume of 2.5% ammonium molybdate, and 1 volume of 10% ascorbic acid. Five milliliters of this reagent was added to the sample solution and to a phosphate standard which had been treated identically. The absorbance of the phosphomolybdate solutions at 820 nm was recorded after a 20.0-min interval from mixing time.

Due to the existence of consecutive reactions which could produce orthophosphate ion, samples for kinetic measurements were only taken up to the point that the concentration of the orthophosphate ion was equal to one-third the initial concentration of triphosphate complex.

Kinetic data at pH 6 or less could not be obtained for the catalyzed reaction of the γ-isomer due to the formation of a precipitate. In the case of the β-isomer, kinetic data at pH greater than 9 was unobtainable due to reduction.

Results

NMR. The ^{31}P NMR spectrum of the γ-isomer at pH 8 shown in Figure 1a has two doublets, 3.54 ($J = 20.0$ Hz) and −3.20 ppm

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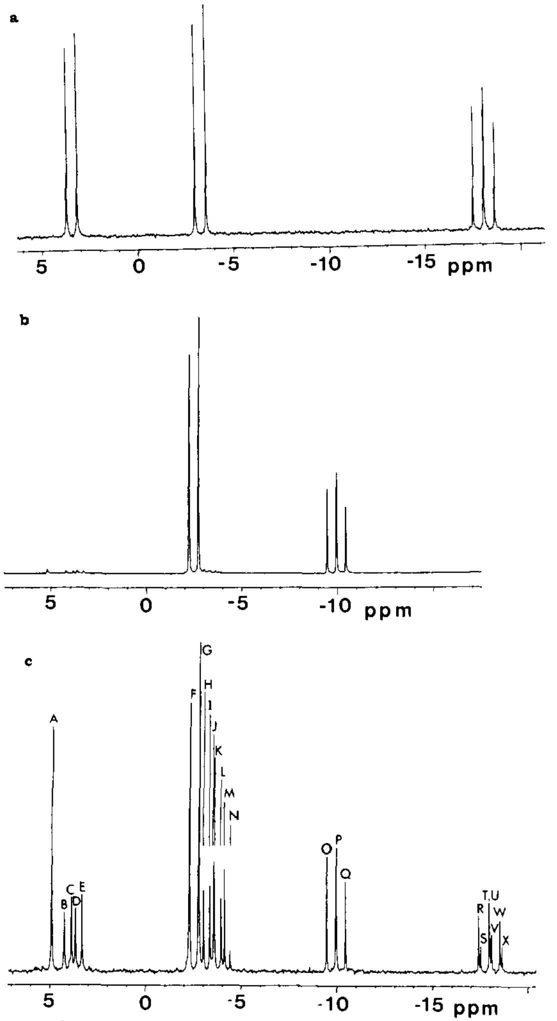

Figure 1. ^{31}P NMR spectrum (36.4 MHz) at pH 8.0 and ambient temperature of the (a) γ-isomer, (b) β-isomer, and (c) β-isomer after a 34-day time lapse at pH 7.4 and ambient temperature.

(J = 21.0 Hz), along with a doublet of doublets at −17.99 ppm. The downfield doublet is attributed to the coordinated γ-phosphate and the upfield doublet is attributed to the α-phosphorus. Each of these phosphates gives rise to a doublet due to the splitting of the signal caused by the β-phosphate. The signal of the β-phosphate is split into a doublet of doublets by the nonequivalent α- and γ-phosphates.

The ^{31}P NMR spectrum of the β-isomer shown in Figure 1b has a doublet at −2.44 ppm (J = 17.6 Hz) and a triplet at −9.89 ppm. The doublet is attributed to the signal of the two equivalent terminal phosphates split by the β-phosphate, and the triplet is the signal of the β-phosphate split by the equivalent terminal phosphates. Scale expansion of the spectrum reveals the triplet to be the doublet of doublets expected from an AB_2 system.[22] The two center peaks in the doublet of doublets have a separation of only 1 Hz.

The spectrum of the β-isomer after a 34-day time lapse is shown in Figure 1c. The β-isomer is seen to have undergone many changes. Peaks F, G, O, P, and Q are due to the β-isomer and peak N is due to free pyrophosphate; the other peaks are due to three processes: (1) hydrolysis to free orthophosphate, singlet (peak A), and pentaamine(pyrophosphato)cobalt(III), two doublets (peaks B, D, I, and L) J = 21.2 Hz; (2) aquation to release free triphosphate, doublet (peaks J and M), J = 20.0 Hz, and triplet (peaks S, V, and X); and (3) isomerization to the γ-isomer, doublet (peaks C and E), J = 19.8 Hz, doublet (peaks H and K), J = 20.7, and double doublet (peaks R, T, U, and W).

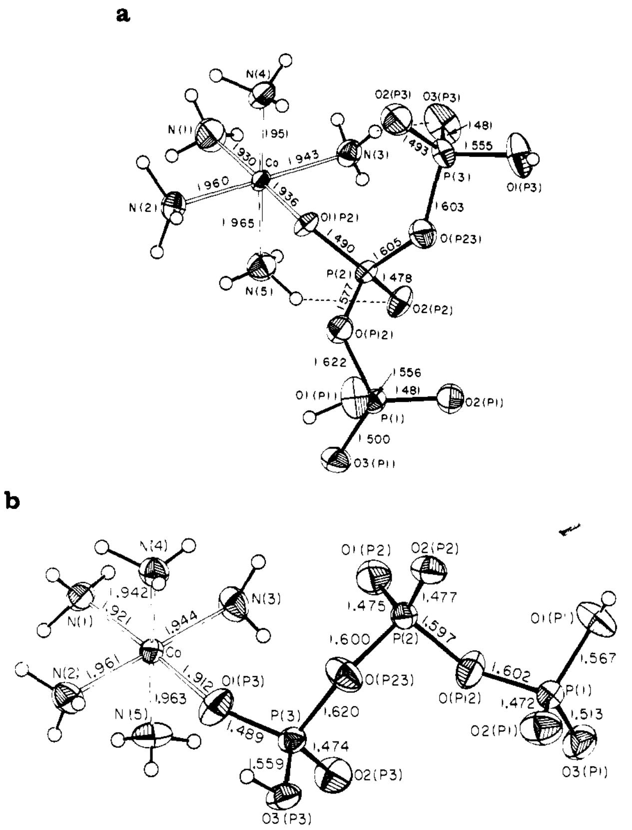

Figure 2. ORTEP drawings of the (a) β-monodentate and (b) γ-monodentate forms of pentaammine(triphosphate)cobalt(III) showing non-hydrogen atoms as 50% thermal probability ellipsoids and hydrogen atoms as spheres of arbitrary size. Atom labeling and non-hydrogen bond lengths are shown.

From the NMR spectrum, an approximate rate constant of 10^{-7} s^{-1} at room temperature has been estimated for the rate of isomerization of the β-isomer to the γ-isomer. In contrast to the β-isomer, the γ-isomer is very stable, with the NMR spectrum being virtually unchanged after 34 days.

Crystal Structures. The atomic coordinates for the two crystal structures are presented in Table II. ORTEP[23] diagrams showing bond lengths between non-hydrogen atoms are given in Figure 2.

The polyphosphate chain of the β-monodentate displays an extended conformation with the P(1) phosphate group rotated away from the metal ion while the P(3) phosphate group is sufficiently close to the pentaamminecobalt(III) moiety to permit intramolecular hydrogen bonding (see below). In contrast, the polyphosphate chain of the γ-monodentate forms a pseudo-left-handed (counterclockwise) helix with the O2(P3)–P(3)–O(P23)–P(2)–O(P12)–P(1)–O2(P1) chain forming a complete turn where the O2(P3) and O2(P1) atoms, which are 4.615 Å apart, overlap when viewed down the helix axis. The P_1–P_3 distance and P_1–P_2–P_3 (PPP) virtual bond angle serve as measures of the degree to which a triphosphate chain is extended or contracted. The P_1–P_3 distance of 4.970 Å and PPP angle of 118° for the β-monodentate isomer are significantly larger than the corresponding values of 4.638 Å and 104° for the γ-monodentate form since the γ-form, which has both torsion angles in the *gauche*⁻ domain, forms a more closely packed helix.

The conformational parameters for these two structures can be compared with the other metal triphosphate complexes.[10] Of the metal-complexed triphosphate structures, the β-monodentate and β,γ-bidentate structures are the most extended, with P_1–P_3 distances of 4.970 and 4.905 Å, respectively, and PPP angles of 118 and 116°, respectively. In both these complexes, the distal

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Table II. (a) Fractional Positional Parameters for All Atoms of β- and γ-Pentaammine(dihydrogen triphosphato)cobalt(III) Monohydrate[a]

atom	x	y	z	B_{eq} or B, Å^2	atom	x	y	z	B_{eq} or B, Å^2
(a) β-Pentaammine(dihydrogen triphosphato)cobalt(III) Monohydrate					(b) γ-Pentaammine(dihydrogen triphosphato)cobalt(III) Monohydrate				
Co	345 (1)	1752 (1)	2206 (1)	1.35 (1)	Co	3981 (1)	7746 (1)	7322 (1)	1.77 (1)
P(1)	5942 (2)	4093 (1)	4040 (1)	1.79 (4)	P(1)	11027 (2)	4364 (1)	6100 (1)	2.30 (4)
O1(P1)	5253 (7)	5100 (3)	4402 (3)	3.01 (11)	O1(P1)	11697 (6)	4416 (3)	5005 (3)	3.72 (11)
O2(P1)	7468 (6)	4219 (3)	3347 (3)	2.62 (10)	O2(P1)	12318 (6)	4761 (3)	6796 (3)	3.79 (11)
O3(P1)	6263 (7)	3418 (3)	4859 (3)	2.73 (10)	O3(P1)	10247 (5)	3344 (3)	6273 (3)	3.30 (9)
O(P12)	4091 (6)	3700 (3)	3508 (3)	2.42 (10)	O(P12)	9320 (5)	5037 (3)	6099 (3)	3.23 (10)
P(2)	3811 (2)	3157 (1)	2534 (1)	1.60 (4)	P(2)	8962 (2)	6198 (1)	5923 (1)	2.02 (4)
O1(P2)	1802 (6)	2896 (3)	2507 (3)	1.98 (9)	O1(P2)	7298 (6)	6261 (3)	5396 (3)	3.40 (9)
O2(P2)	5185 (6)	2374 (3)	2374 (3)	2.50 (9)	O2(P2)	10477 (6)	6710 (3)	5496 (3)	2.79 (9)
O(P23)	4168 (7)	4042 (3)	1814 (3)	2.84 (10)	O(P23)	8786 (5)	6620 (3)	7027 (3)	3.23 (9)
P(3)	3187 (3)	4249 (1)	814 (1)	2.04 (4)	P(3)	7396 (2)	6430 (1)	7904 (1)	2.00 (4)
O1(P3)	4701 (8)	4841 (3)	279 (3)	3.63 (11)	O1(P3)	6086 (5)	7242 (3)	7864 (3)	3.29 (9)
O2(P3)	1501 (7)	4849 (3)	1010 (3)	3.23 (11)	O2(P3)	6779 (5)	5390 (3)	7874 (3)	3.39 (9)
O3(P3)	2881 (8)	3322 (3)	301 (3)	3.59 (12)	O3(P3)	8558 (5)	6627 (3)	8826 (3)	2.76 (9)
N(1)	−1221 (9)	644 (4)	1942 (4)	3.53 (15)	N(1)	1887 (6)	8339 (3)	6842 (3)	2.39 (9)
N(2)	−1113 (7)	2051 (3)	3342 (3)	2.19 (10)	N(2)	3904 (7)	8627 (3)	8483 (3)	2.98 (10)
N(3)	1799 (8)	1523 (3)	1068 (3)	2.17 (10)	N(3)	4061 (7)	6884 (3)	6164 (3)	2.82 (9)
N(4)	−1245 (7)	2621 (4)	1472 (4)	2.59 (13)	N(4)	5379 (6)	8747 (3)	6661 (3)	2.36 (9)
N(5)	2012 (8)	892 (3)	2924 (3)	2.58 (12)	N(5)	2684 (7)	6707 (4)	8028 (4)	3.07 (13)
O(W)	5234 (8)	6625 (3)	453 (4)	4.23 (13)	O(W)	10189 (6)	4935 (4)	9607 (4)	4.31 (11)
H1(N1)	−162 (10)	31 (4)	244 (4)	4.0	H1(N1)	86 (7)	827 (5)	732 (5)	4.0
H2(N1)	−233 (10)	83 (4)	173 (4)	4.0	H2(N1)	165 (6)	801 (4)	631 (5)	4.0
H3(N1)	−57 (10)	17 (4)	159 (4)	4.0	H3(N1)	196 (7)	892 (4)	682 (5)	4.0
H1(N2)	−251 (9)	209 (4)	316 (5)	4.0	H1(N2)	487 (7)	880 (4)	865 (5)	4.0
H2(N2)	−96 (10)	255 (4)	355 (4)	4.0	H2(N2)	327 (6)	924 (4)	840 (5)	4.0
H3(N2)	−60 (10)	166 (5)	389 (4)	4.0	H3(N2)	341 (6)	840 (4)	891 (5)	4.0
H1(N3)	106 (10)	121 (4)	74 (4)	4.0	H1(N3)	456 (7)	644 (4)	639 (5)	4.0
H2(N3)	186 (10)	208 (5)	72 (5)	4.0	H2(N3)	310 (7)	664 (4)	591 (5)	4.0
H3(N3)	258 (11)	129 (5)	117 (4)	4.0	H3(N3)	487 (8)	711 (5)	564 (5)	4.0
H1(N4)	−253 (9)	259 (4)	168 (5)	4.0	H1(N4)	648 (6)	901 (4)	696 (5)	4.0
H1(N4)	−77 (10)	319 (4)	153 (4)	4.0	H2(N4)	574 (8)	855 (4)	616 (5)	4.0
H2(N4)	−130 (10)	248 (4)	86 (5)	4.0	H3(N4)	455 (7)	936 (4)	662 (5)	4.0
H3(N4)	156 (10)	81 (4)	341 (4)	4.0	H1(N5)	257 (8)	602 (5)	776 (5)	4.0
H1(N5)	322 (10)	110 (4)	300 (4)	4.0	H2(N5)	180 (7)	684 (4)	818 (5)	4.0
H2(N5)	230 (10)	27 (4)	254 (5)	4.0	H3(N5)	320 (7)	670 (4)	852 (5)	4.0
H3(N5)	463 (10)	505 (5)	501 (4)	4.0	H(O1P1)	1263 (9)	472 (5)	484 (5)	4.0
H(O1P1)	490	549	34	4.0	H(O3P3)	850 (6)	727 (4)	887 (5)	4.0
H(O1P3)	671 (10)	661 (5)	25 (4)	4.0	H1(W)	916 (8)	445 (4)	992 (5)	4.0
H1(W)	461 (10)	673 (5)	−4 (4)	4.0	H2(W)	960 (7)	546 (4)	936 (4)	4.0
H2(W)									

[a] Values are multiplied by 10^4 for non-hydrogen atoms and 10^3 for hydrogen atoms.

phosphate group exhibits neither coordination nor hydrogen bonding to the metal. The α,β,γ-tridentate complex is more compressed, since the metal is coordinated to all three phosphate moieties, with a P_1–P_3 distance of 4.213 Å and a PPP angle of 93°. The α,γ-bidentate complex is the most compressed since the absence of a coordination bond to the β-phosphate group permits the α- and γ-phosphate groups to be drawn closer together; the P_1–P_3 distance is 3.987 Å and the PPP angle is 86°.

The bridge P–O bond lengths differ by 0.045 Å at O(P12) in the β-isomer [1.577 (5) vs. 1.622 (5) Å] and differ by 0.02 Å at O(P23) in the γ-isomer [1.600 (5) vs. 1.620 (5) Å]. In contrast, the P–O bonds at O(P23) of the β-isomer and O(P12) of the γ-isomer are nearly equal. The other P–O distances (combined for both structures) have means and standard deviations of 1.559 ± 5 and 1.485 ± 12 Å for the protonated and unprotonated oxygen atoms, respectively. These values are comparable to those reported for the other triphosphate complexes.[8-10] The Co–O coordination distance for the β-isomer is 0.024 Å longer than for the γ-isomer [1.936 (4) vs. 1.912 (4) Å]. Of the ammonia ligand coordination distances, the Co–N(1) distance opposite to the Co–O linkage is the shortest in both structures (1.930 and 1.921 Å for the β and γ forms, respectively). The observed differences in the other ammonia coordination distances (average of 1.954 ± 10 Å for both structures) may be partially attributed to the differences in their hydrogen bonding environments.

A list of hydrogen bonding interactions for both structures is given in Table III. In the β-monodentate form, the strongest hydrogen bond occurs between O1(P3) and the water of hydration with an oxygen–oxygen distance of 2.492 Å. There is also a hydrogen bonding interaction between O1(P1) and O2(P3) with a length of 2.598 Å. Of the 15 ammonia protons, 12 engage in hydrogen bonding. Two of these are intramolecular: N(3) to O3(P3) and N(5) to O2(P2) with lengths of 2.808 and 3.156 Å, respectively. A similar intramolecular hydrogen bonding scheme was observed for the structure of monodentate $CoHP_2O_7(NH_3)_5\cdot H_2O$, which can be visualized as being the β-monodentate isomer with one of the terminal phosphate groups removed.[8] In the γ-monodentate structure, the strongest hydrogen bond between the proton of O3(P3) and O3(P1) results in a short O3(P3)–O3(P1) distance of only 2.483 Å. This is the only direct interaction between phosphate groups; all the others are mediated either by the ammonia ligands or by the water molecule. This short hydrogen bond contributes to the lengthening of the P(1)–O3(P1) distance by 0.03 Å when compared to the other nonprotonated P–O bond lengths. Two of the eleven hydrogen-bonded ammonia protons form bifurcated hydrogen bonds: H2(N1) forms hydrogen bonding interactions with both O1(P2) and O2(P2) of different symmetry-related molecules while H2(N2) forms hydrogen bonds to O(P12) and O2(P3) of separate molecules.

Acid Ionization Constants. From potentiometric titrations, the γ-isomer was calculated[24] to have a pK_1 of 3.45 (5) and a pK_2 of 6.70 (2) at 25 °C and a pK_1 of 3.49 (5) and a pK_2 of 6.72 (5) at 40 °C. The β-isomer had pK values with a difference of less

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Table III. List of Hydrogen Bonding Interactions in the Crystal Structures

hydrogen bond A–H···B	symmetry code	translation[a]			distances, Å			angle, deg
		x	y	z	A–H	H···B	A···B	A–H···B
β-Monodentate Isomer								
N(1)–H1(N1)···O2(P3)	4	0	−1	0	0.89	2.28	3.096	153
N(2)–H1(N2)···O2(P2)	1	−1	0	0	1.04	2.03	3.027	159
N(2)–H2(N2)···O2(P1)	1	−1	0	0	0.75	2.57	3.148	135
N(2)–H3(N2)···O3(P1)	3	−1	0	1	1.01	2.22	3.128	149
N(2)–H3(N2)···O2(P3)	4	0	−1	0	1.01	2.57	3.172	118
N(3)–H1(N3)···O1(P3)	3	−1	0	0	0.83	2.26	3.067	165
N(3)–H2(N3)···O3(P3)	1	0	0	0	0.91	1.95	2.808	157
N(3)–H3(N3)···O1(P1)	4	1	−1	0	0.66	2.40	2.960	144
N(4)–H1(N4)···O2(P2)	1	−1	0	0	0.97	1.94	2.888	165
N(4)–H3(N4)···O3(P3)	3	−1	0	0	0.89	2.06	2.887	155
N(5)–H2(N5)···O2(P2)	1	0	0	0	0.92	2.42	3.156	137
N(5)–H2(N5)···O(W)	4	1	−1	0	0.92	2.55	3.192	127
N(5)–H3(N5)···O2(P1)	4	1	−1	0	1.03	1.92	2.939	169
O1(P1)–H(O1P1)···O2(P3)	2	0	1	0	0.97	1.64	2.598	171
O1(P3)–H(O1P3)···O(W)	1	0	0	0	0.91	1.59	2.492	180
O(W)–H1(W)···O3(P1)	2	1	1	−1	1.10	1.56	2.658	174
O(W)–H2(W)···O3(P1)	4	1	0	0	0.84	2.42	2.724	102
γ-Monodentate Isomer								
O1(P1)–H(O1P1)···O(W)	2	2	1	−1	0.86	1.78	2.623	165
O3(P3)–H(O3P3)···O3(P1)	4	2	0	1	0.86	1.75	2.483	141
N(1)–H1(N1)···O3(P1)	4	1	0	1	1.03	2.09	3.034	152
N(1)–H2(N1)···O2(P2)	1	−1	0	0	0.86	2.25	3.043	153
N(1)–H2(N1)···O1(P2)	3	−1	1	1	0.86	2.55	3.083	121
N(1)–H3(N1)···O2(P3)	4	1	0	1	0.79	2.23	2.960	155
N(2)–H2(N2)···O2(P3)	4	1	0	1	0.96	2.31	3.034	132
N(2)–H2(N2)···O(P12)	4	1	0	1	0.96	2.38	3.184	141
N(3)–H2(N3)···O2(P2)	1	−1	0	0	0.89	2.11	2.932	154
N(3)–H3(N3)···O2(P2)	3	−1	1	1	0.99	2.25	3.126	146
N(4)–H1(N4)···O2(P1)	4	2	0	1	1.01	2.17	3.062	146
N(4)–H2(N4)···O2(P2)	3	−1	1	1	0.78	2.27	2.974	151
N(4)–H3(N4)···O2(P3)	4	1	0	1	1.05	1.85	2.835	156
N(5)–H1(N5)···O2(P1)	1	−1	0	0	1.00	2.13	3.105	164
N(5)–H3(N5)···O1(P1)	2	1	1	0	0.77	2.50	3.099	135
O(W)–H1(W)···O1(P2)	2	1	1	0	1.11	1.61	2.725	179
O(W)–H2(W)···O3(P3)	1	0	0	0	0.90	1.90	2.801	175

[a] Symmetry codes: (1) x, y, z; (2) $^1/_2 - x, -y, ^1/_2 + z$; (3) $^1/_2 + x, ^1/_2 - y, -z$; (4) $-x, ^1/_2 + y, ^1/_2 - z$.

Table IV. Rate Constants as a Function of pH for Cobalt(III) Triphosphato[a] Complexes at 40 °C and $I = 1.0$ ($\times 10^5$ s^{-1})

pH	β-isomer uncatalyzed	β-isomer catalyzed[b]	γ-isomer uncatalyzed	γ-isomer catalyzed[b]
7.0		15		
7.4				<1.0
8.0		24		
9.0	<0.1	42	<0.001	<0.7

[a] Solution are 25 mM in cobalt(III) triphosphato species. [b] Catalyzed reactions are 20 mM in *cis*-[Co(cyclen)(OH$_2$)$_2$]$^+$.

than 2.7 pH units so the Noyes equation[25] was employed to calculate the pK values of this isomer. At 25 °C the β-isomer has a pK_1 of 4.37 (4) and a pK_2 of 6.12 (5).

Hydrolysis Rate Constants. Since pseudo-first-order conditions were employed for all kinetic experiments, rate constants k_{obsd} are defined by eq 1.

$$+\mathrm{d}[\text{total orthophosphate}]/\mathrm{d}t = k_{\mathrm{obsd}}[\text{phosphato complex}] \quad (1)$$

Table IV displays the rate constants for phosphate hydrolysis at various pH values. Under all conditions examined, the β-isomer hydrolyzes faster than the γ-isomer. In the cyclen-catalyzed reactions, the rate of hydrolysis of each isomer increases with increasing pH. At pH 9, the isomers underwent decomposition during the time frame required for the reaction.

Only upper limits for rate constants are given for the uncatalyzed reaction of each isomer and for the catalyzed reaction of the γ-isomer at pH 7.4; these hydrolysis reactions were so slow that aquation and isomerization reactions may have occurred during the time necessary to observe hydrolysis products.

Acid/Base Consumption. During the course of the *cis*-[Co(cyclen)(OH)$_2$]$^+$ catalyzed experiments, the pH, the acid consumption, and the base consumption were recorded.

When the solution containing the β-isomer at pH 7.0 was mixed with the solution containing the *cis*-[Co(cyclen)(OH)$_2$]$^+$ at pH 9.0, the pH rose rapidly to 10.6. An experiment which measured the amount of nitric acid needed to lower the pH back to 9.0 required 1.06 mol of acid for each mol of *cis*-[Co(cyclen)(OH)$_2$]$^+$ present. After the initial addition of acid, rapid base consumption began and continued for the duration of the reaction. When the same experiment was carried out at pH 8.0, the amount of acid initially consumed was the same as that amount observed at pH 9.0, but the subsequent base consumption occurred at a slightly slower rate than the rate of base consumption at pH 9.0.

In the reaction of the γ-isomer at pH 9.0, the pH rose to 11 when the solution containing the γ-isomer was mixed with the solution containing *cis*-[Co(cylen)(OH)$_2$]$^+$. The amount of nitric acid required to lower the pH back to 9.0 was equivalent to 1.15 mol of acid for each mol of *cis*-[Co(cyclen)(OH)$_2$]$^+$ present. After the initial addition of acid, acid consumption slowly occurred for a period of approximately 80 min from mixing time. At approximately 90 min from mixing time, slow base consumption began and continued for the duration of the experiment. When the reaction of the γ-isomer was carried out at pH 7.4, the amount of acid initially consumed upon mixing was the same as the amount observed at pH 9.0. After the pH was lowered back to 7.4, a slight amount of acid was consumed but after about 20 min from mixing time, the acid consumption discontinued. The pH then remained at 7.4 with no detectable acid or base consumption. The experiment was discontinued 3 h after mixing time. Very little hydrolysis occurred during the time of this experiment.

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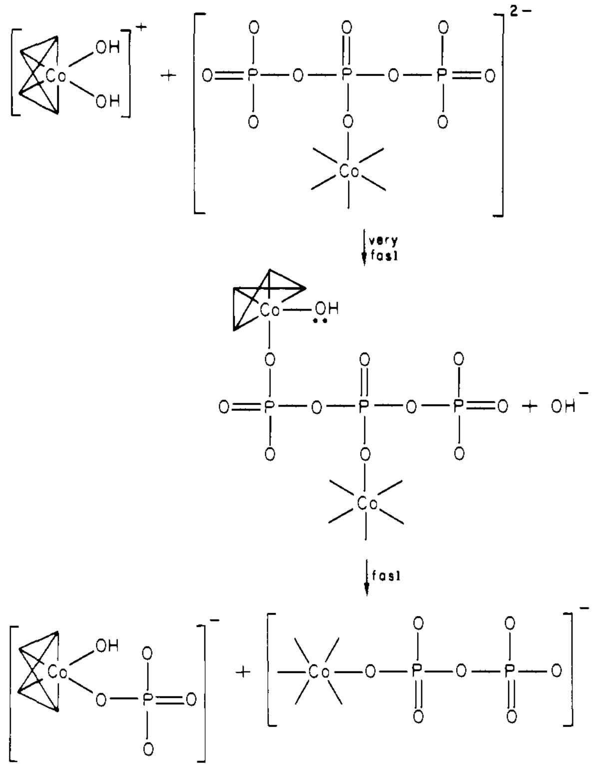
Scheme I. Proposed mechanism for the phosphate hydrolysis of β-$[Co(NH_3)_5P_3O_{10}]^{2-}$ with *cis*-$[Co(cyclen)(OH)_2]^+$ at pH 9.0



Discussion

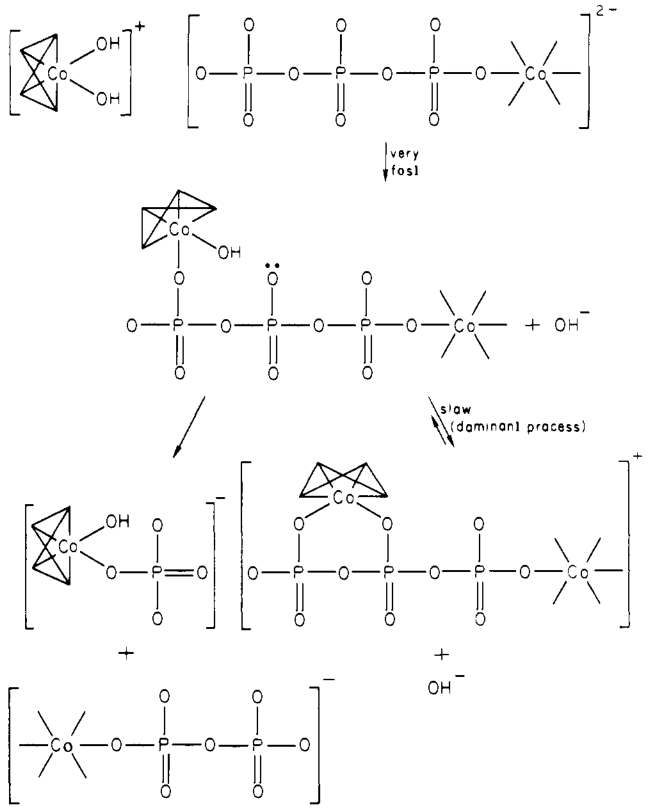
The β- and γ-isomers of $Co(NH_3)_5H_2P_3O_{10}$ have been definitively characterized by X-ray crystallography in the crystal and by ^{31}P NMR spectroscopy in solution. Both complexes are sufficiently stable that the reactions of each one can be examined independently of the other. The γ-isomer in solution is more stable with respect to hydrolysis, aquation, and isomerization than the β-isomer. The explanation for the greater stability of the γ-isomer may lie in the greater basicity of the γ-phosphate group over the β-phosphate group.[26,27] It is well established[28] that the rates of base hydrolysis of pentaamminecobalt(III) complexes decrease as the basic strength of the leaving group increases. The fact that the Co–O bond of the γ-isomer is 0.024 Å shorter than the corresponding bond of the β-isomer is consistent with the greater stability of the γ-isomer.

Catalyzed Kinetics. The results of experiments catalyzed by *cis*-$[Co(cyclen)(OH)_2]^+$ show a striking difference in the rates of hydrolysis of the two isomers (Table IV). At pH 9.0 the β-isomer is hydrolyzed 50 times faster than the γ-isomer. This difference can be explained by a mechanism involving competition between hydrolysis and chelation reactions.

When a solution containing either the β-isomer or the γ-isomer is mixed with a solution containing a limiting amount of the cyclen complex at pH 9, 1 mol of hydroxide ions for each mol of the cyclen complex is released within the time of mixing. Since no phosphate is hydrolyzed during this time, the initial release of base must be due to (monodentate) coordination of a free terminal phosphate of the β- or γ-isomer to *cis*-$[Co(cyclen)(OH)_2]^+$ causing this complex to release one of its hydroxide groups.

For the β-isomer, the initial release of base is followed by the rapid consumption of base while the phosphate hydrolysis reaction occurs. The β-phosphate is coordinated to the electron-withdrawing cobalt(III) center, and therefore the nucleophilic character of the β-phosphate is decreased. The remaining *cis*-hydroxide ion on the cobalt(III) cyclen complex is a stronger nucleophile than the β-phosphate and attacks the β-phosphorus, causing the rapid hydrolytic reaction as shown in Scheme I.

Scheme II. Chelation vs. hydrolysis of γ-$[Co(NH_3)_5P_3O_{10}]^{2-}$ with *cis*-$[Co(cyclen)(OH)_2]^+$ at pH 9.0



For the γ-isomer, the initial release of base is followed by a slower base-producing reaction. The slow production of base is apparently due to the release of the second hydroxide ion by the cyclen complex as it chelates to the β-phosphate. Chelation occurs instead of hydrolysis because the nucleophilic character of the β-phosphate is not decreased by coordination to an electron-withdrawing cobalt(III) center. In contrast to the case for the β-isomer, the β-phosphate is the attacking nucleophile and thus chelation to the cyclen complex occurs as shown in Scheme II. After an equilibrium for the chelation reaction is reached, slow base consumption is observed as a result of the hydrolysis reaction.

The change in the rate of hydrolysis for each isomer in the pH range 7–9 suggests that decomposition of the *cis*-hydroxide ion of the cyclen complex is a prerequisite to catalyzed hydrolysis. This mechanistic step has previously been shown to apply in a related system[13] using the bidentate complex β,γ-$Co(NH_3)_4H_2P_3O_{10}$.

Analogy to ATP Systems. NMR studies have indicated that Mg^{2+} will preferentially bind polyphosphates to form 6-membered chelate rings as in α,β-bidentate Mg-ADP or in β,γ-bidentate Mg-ATP.[29,30] In solution, the screw sense isomers in Mg-ATP can undergo interconversion via a γ-monodentate intermediate. On the enzyme active site, however, usually only one stereoisomer for bidentate metal binding is permitted, with some enzymes requiring the left-handed isomer and others the right-handed isomer.[6,7] Since Mg^{2+} binding in solution is nonstereospecific while the enzyme binding sites are very stereospecific, this interconversion restores the concentration of the active stereoisomer concurrent with its depletion by the enzyme-catalyzed reaction. This process may be significant during enzyme–substrate binding as the polyphosphate chain is adjusted to fit the steric constraints of the active site. Models of γ-monodentate Co-ATP are presented in Figure 3. The base of AMP is *anti* and the ψ torsion angle

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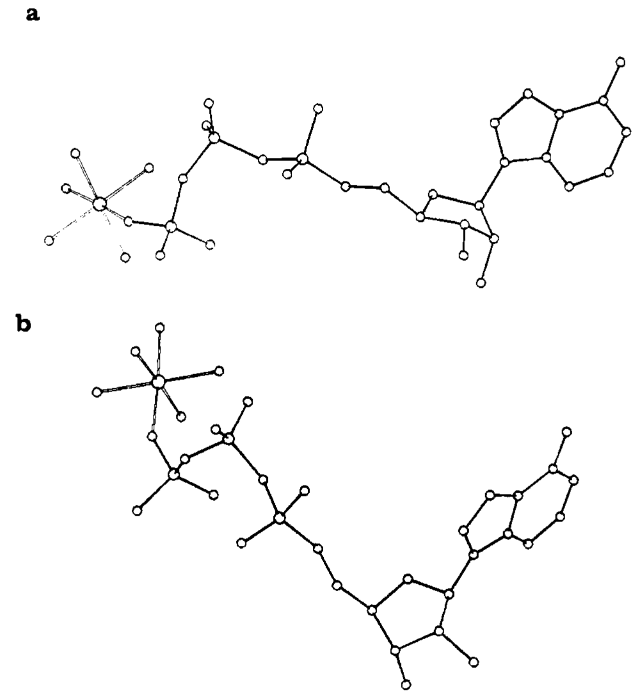

Figure 3. Models for possible γ-monodentate metal coordination to ATP: (a) ribose ring with 3′-endo pucker; (b) ribose ring with 2′-endo pucker.

[C(3′)–C(4′)–C(5′)–O(5′)] is set to the *trans* domain which was shown to be the preferred conformation for several enzymes.[31] Figure 3a depicts the ribose ring in the 3′-endo conformation (based on the coordinates for the monoclinic structure of 5′-AMP[32]) while Figure 3b shows a 2′-endo ribose pucker (based on the orthorhomibc 5′-AMP structure[33]). Structures such as those depicted in Figure 3, using Mg^{2+} as the metal cofactor, could be transiently formed at the enzyme active site as the metal ion forms the required bidentate coordination mode prior to catalysis and may also be transiently present after catalysis during metal dissociation from the product. Notice that the ribose pucker significantly affects the orientation of the polyphosphate chain relative to the base. Thus the enzyme not only demands a specific screw sense isomer of the bidentate metal–polyphosphate coordination complex but may also apply severe restrictions on the allowed puckering mode for the ribofuranose ring.

Kinetic studies[1] on the mechanism of phosphoribosylphosphate synthetase have indicated that the enzyme requires the complex Mg–ATP as an active substrate and free magnesium as an essential activator. Mildvan and Switzer[34] performed studies on the hydrolysis of β,γ-$[Co(NH_3)_4ATP]^-$ in the presence of the enzyme, magnesium(II), and manganese(II). They showed β,γ-$[Co(NH_3)_4ATP]^-$ to exhibit substrate activity only in the presence of a divalent cation. The CD spectrum of isolated product, $[Co(NH_3)_4(PPR)]^{2-}$ (PPR = pyrophosphorylribose 5-phosphate), indicated an inversion about the β-phosphate atom. This inversion suggests that the β-phosphate group is activated by the nucleotide-bound divalent cation. The present work indicates that the role of the metal cation coordinated at ATP is to decrease the nucleophilicity of the β-phosphate. The mode of coordination to the ATP could be β or β,γ. The lability of magnesium(II) would make either or both modes of coordination to ATP possible. The β-coordination mode would leave the nucleophilic character of the γ-phosphate intact, while the β,γ-coordination would facilitate the nucleophilic attack upon the γ-phosphate.

Acknowledgment. We are grateful to Professor Ronald Viola of Southern Illinois University, Edwardsville, IL, for providing NMR spectra. This research was supported by a grant from the National Institutes of Health to M.S. (GM-17378) and a Research Corporation grant and a National Science Foundation grant (CHE-8024043) to R.D.C.

Registry No. β-$Co(NH_3)_5H_2P_3O_{10}\cdot H_2O$, 89530-73-4; γ-$Co(NH_3)_5H_2P_3O_{10}\cdot H_2O$, 89530-74-5; $[Co(NH_3)_5CO_3]NO_3$, 15244-74-3; *cis*-$[Co(cyclen)(OH)_2]^+$, 89530-75-6; triphosphate, 14127-68-5; dihydrogen triphosphate, 89556-19-4.

Supplementary Material Available: A table giving anisotropic temperature factors for non-hydrogen atoms and a list of all observed and calculated structure factors for both structures (10 pages). Ordering information is given on any current masthead page.

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