probably an upper limit, for the kinetics of disappearance of MbO_2 and MbCO, independent of the excitation energy. As can be seen from Figure 2, excitation at 355 nm results in dissociation from both channels while 530-nm excitation can utilize only one channel and, therefore, results in a lower quantum yield.

If the charge-transfer manifold in oxyheme were not coupled to the ground state, the dissociative nature of these states would have resulted in quantum yield of unity. Indeed the existence of a nonradiative decay channel, as shown in Figure 2, together with the dissociative nature of the charge-transfer states, is responsible for the simultaneous ready photolability and low quantum yield of oxyhemoproteins. Assuming only a single dissociating charge-transfer state below the Q band and also assuming simple exponential kinetics for both dissociation and decay out of this state, we calculate[41] a quantum yield of 0.25 if we assume dissociation and decay lifetime values of 6 and 4 ps (see Figure 2), respectively. This value is not far from the observed value of about 0.1. While neither value is accurately determined, the difference may indicate that the lower dissociation channel in oxyheme is more complicated than a single dissociating state and that the decay mechanism is not a simple exponential. The same simple model also gives a lifetime of about 2.4 ps for the depletion of the charge-transfer state. This provides an explanation for the observation of Shank et al.[19] that their excitation of HbO_2 at 615 nm resulted in an induced signal that decayed with a time constant of 2.5 ps. If we consider the 2.5 ps as a lower limit to the depletion of the dissociating state, it follows that neither the dissociation nor the decay lifetime can be less than 2.5 ps, and therefore, the 0.5-ps rise time of their induced signal cannot be a lower limit to photodissociation lifetime in HbO_2. We thus tend to agree with the suggestion[20] that HbO_2 was excited into a dissociating state by the 615-nm subpicosecond pulse used, and the new species was monitored until depletion.

Finally we should point out that our results are at variance with the conclusion reached by Hoffman and Gibson[28] that the low quantum yield of HbO_2 is due to radiationless decay to charge-transfer states which they consider both short lived and stable. We rather assert that these states themselves are photodissociating and that the low quantum yield of HbO_2 results from the competing dissociation and decay kinetics which holds true for both upper and lower channels.

Conclusion

Single configuration interaction calculations of the excited states of oxyheme complex as a function of iron–ligand distance have been made and the results used to establish that the photodissociation of oxyhemoproteins occurs through two independent channels, one above and one below the Q band in energy. The upper channel involves $d\pi \rightarrow d_{z^2}{}^*$ photoexcited states and thus resembles the only dissociation channel available to carbon monoxyhemoproteins, most likely with similar kinetics. The lower channel involves charge-transfer excited states and is specific for oxyhemoproteins. Mechanisms have been proposed for the photodissociation of O_2 and CO which are consistent with the picosecond experimental data. It is found that the low quantum yield of oxyhemoprotein at low excitation energies is due to competitive decay from dissociative charge-transfer states and at high excitation energies is due to nonradiative decay to singlet states below the dissociative $d\pi \rightarrow d_{z^2}{}^*$ channel.

Acknowledgment. We gratefully acknowledge financial support for this work from NSF Grant PCM-7921591. We also thank Dr. Dale Spangler for many helpful discussions during the course of this work and Brenda Wells for help in preparation of the manuscript.

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(41) Assuming a simple two-state model below the Q band, and assuming simultaneous exponential dissociation to an intermediate state I with a lifetime of $\tau_1 = 6$ ps and a decay to the ground state with a lifetime of $\tau_2 = 4$ ps, similar to that shown in Figure 2 of the text, we can write

$$n(t) = n_0 \exp(-t/\tau_{\text{dep}});\ \tau_{\text{dep}} = \tau_1\tau_2/(\tau_1 + \tau_2)$$

where n_0 is the population of the initially excited states near the saturation level and $n(t)$ is the population at time t with depletion time constant of $\tau_{\text{dep}} = 2.4$ ps. If $n_1(t)$ is the population of the intermediate species resulting from dissociation, it is straightforward to show that

$$n_1(t) = (n_0\tau_{\text{dep}}/\tau_1)[1 - \exp(-t/\tau_{\text{dep}})]$$

The quantum yield of dissociation in the time scale of depletion lifetime of the dissociating state is given by

$$\phi = n_1(\tau_{\text{dep}})/n_0 = \tau_1(1 - e^{-1})/(\tau_1 + \tau_2) = 0.25$$

for $\tau_1 = 6$ and $\tau_2 = 4$ ps.

Mechanism of Cobalt(III)-Promoted Hydrolysis of Triphosphate Ion

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Contribution from the Department of Chemistry, Wichita State University, Wichita, Kansas 67208. *Received July 15, 1981*

Abstract: The mechanism of hydrolysis of phosphates has been studied by using the well-characterized complex β,γ-[$Co(NH_3)_4H_2P_3O_{10}$], in which the triphosphate ion is coordinated as a bidentate ligand. The rate of hydrolysis of the triphosphate ion to pyrophosphate ion in this complex has been studied in the presence of the cobalt(III) complex of the macrocyclic tetraamine ligand, cyclen. In the presence of this macrocyclic complex, the rate of hydrolysis was increased by 5×10^5 over the rate for the free triphosphate ion. The dependence of the rate upon pH showed that a deprotonation step with a pK of 7.9 was critical for the accelerated hydrolysis to occur. The results are consistent with a mechanism involving coordination of the free end of the phosphate chain in the $Co(NH_3)_4H_2P_3O_{10}$ by the cyclen complex to form a dinuclear species, followed by internal attack on the phosphate chain by a coordinated hydroxide ion.

Phosphates play a vital role in the energy-transfer reactions of living systems, and as a result, an understanding of their chemistry, especially hydrolytic reactions, is of fundamental importance. The ability of coordinated metal ions, particularly cobalt(III), to catalyze the hydrolysis of both inorganic and biological phosphates has been demonstrated by several groups of workers.[1-4] A variety of different effects has been suggested[5] to account for these accelerations, and the purpose of the research

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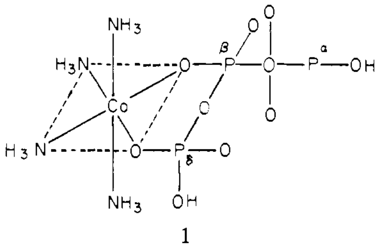
described in this paper is to investigate more fully one of these effects, namely, the simultaneous coordination of a phosphate moiety and an attacking nucleophile, in this case water or hydroxide ion, followed by internal nucleophilic attack. The phosphate-containing compound investigated in this research is the substitution-inert complex β,γ-[Co(NH$_3$)$_4$H$_2$P$_3$O$_{10}$],[6] abbreviated throughout this paper as β,γ complex, **1**. This compound has been characterized by ^{31}P NMR spectroscopy and X-ray crystallography.[7] Use of the β,γ complex greatly facilitates chemical studies in solution, as the nature of the initial cobalt phosphate species is known definitively and does not have to be inferred from NMR or equilibrium data.



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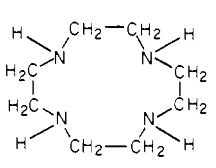

2

Earlier work in this laboratory[8] on the hydrolysis of the β,γ complex promoted by cobalt–amine complexes utilized the compound [Co(NH$_3$)$_4$(H$_2$O)$_2$]$^{3+}$ as the assisting cobalt species. Unfortunately, this complex does not exist in a pure isomeric form but as an equilibrium mixture between the cis and trans froms:

$$\textit{cis}\text{-}[\mathrm{Co(NH_3)_4(H_2O)_2}]^{3+} \rightleftarrows \textit{trans}\text{-}[\mathrm{Co(NH_3)_4(H_2O)_2}]^{3+} \quad (1)$$

The value of the equilibrium constant in this system has been determined to be 6.0 ± 0.5 at both 20 and 30 °C.[9] The problems of interpretation of results from systems in which the assisting complex has multiple forms can be eliminated by utilizing tetraaza macrocyclic ligand complexes where the geometry of the overall complex can be fixed by use of a suitable (N$_4$) donor system.

The majority of the experiments described in this report were carried out by using the diaquo species of the cobalt(III) complex of cyclen, **2** (1,4,7,10-tetraazacyclotetradodecane). The use of cyclen, a 12-membered saturated tetradentate ligand, forces 100% cis geometry on the remaining two coordination sites in an octahedral cobalt(III) complex.[10] In addition to fixing the geometry of the assisting cobalt species, the cyclen complexes of cobalt(III) are stable at high pH. As a result of this stability, experimental data can be taken at pH values in excess of 8.0, conditions under which reduction of cobalt(III) occurs when tetrammine complexes are used.

Experimental Section

General Procedures. All chemicals used were reagent grade except where otherwise stated. Cation and anion resins used were Bio-Rad analytical grade, 100–200 mesh, AG-50W-X2 and AG-1-X2, respectively. All pH measurements were made on an Orion Model 701A digital pH meter fitted with a research grade microcombination electrode. The pH meter was standardized with the appropriate NBS buffer solution at 40 °C. Visible and UV measurements were carried out on a Cary 219 spectrophotometer. Cobalt analyses were carried out by spectrophotometric determination of [CoCl$_4$]$^{2-}$ as previously described.[11] In the case of the ammine complexes, the reduction step required in this determination was carried out by using a 10% solution of SnCl$_2$ in concentrated HCl. Sodium borohydride (10 mg in 2 mL) was used to reduce the less reactive cyclen complexes. Carbon, hydrogen, and nitrogen analyses were performed by Galbraith Labs, Inc., Knoxville, TN.

Syntheses. β,γ-[Co(NH$_3$)$_4$H$_2$P$_3$O$_{10}$]·H$_2$O, 1. This compound was synthesized as previously described by the reaction of [Co(NH$_3$)$_4$CO$_3$]NO$_3$·0.5H$_2$O with sodium triphosphate (Na$_5$P$_3$O$_{10}$) followed by separation of the products by ion-exchange chromatography. The carbonato tetraammine starting material was prepared by the method outlined by Schlessinger.[12]

1,4,7,10,-Tetraazacyclotetradodecane, 2 (cyclen), and Cobalt(III) Complexes. The ligand cyclen tetrahydrochloride was prepared by the method described in the literature,[13] and the complex *cis*-[Co(cyclen)Cl$_2$]Cl via the *cis*-[Co(cyclen)(NO$_2$)$_2$]Cl compound by the method of Collman and Schneider.[10] The catalytically active species *cis*-[Co(cyclen)(H$_2$O)$_2$]$^{3+}$ was prepared either by the base hydrolysis of the dichloro complex, which is very rapid,[14] or by passing a solution of the dichloro complex down an anion-exchange column in the hydroxide from and adjusting the eluate to a suitable pH with nitric acid.[15] The ligand Me$_6$[14]diene dihydrobromide, **3**, was made by the condensation of acetone with 1,2-diaminoethane monohydrobromide[16] using the procedure of Hay, Lawrence, and Curtis. The complex *trans*-[Co(Me$_6$[14]diene)Cl$_2$]$^+$ was derived via the "carbonato route"[17] used by Endicott and co-workers. The diaquo species was generated in solution by base hydrolysis of the dichloro complex.

***C-meso*-5,7,7,12,14,14-Hexamethyl-1,4,8,11-tetraazacyclotetradecane, 4 (tet$_a$), and Cobalt(III) Complexes.** The ligand tet$_a$ was made by borohydride reduction of Me$_6$[14]diene·2HBr followed by a fractional crystallization of the C-racemic and C-meso products.[16] The *trans*-[Co(tet$_a$)Cl$_2$]Cl complex was made as described in the literature,[18] and the *trans*-[Co(tet$_a$)(H$_2$O)$_2$](NO$_3$)$_3$ salt was synthesized by elution of an aqueous solution of the dichloro complex from an anion-exchange column in the hydroxide ion form, followed by acidification of the eluate with an equal volume of 5 M nitric acid. Crystallization occurred overnight at room temperature. The purity of the macrocyclic complexes was checked by elemental analysis.

[Co(NH$_3$)$_4$(H$_2$O)PO$_4$]. This complex was prepared as described in the literature.[19]

Kinetics. Hydrolysis of the β,γ complex was followed by monitoring total production of orthophosphate ion by a method described previously.[1] The technique consists of reducing the cobalt(III) species with acidic vanadium(II), removing labile cations on a cation-exchange column, and then analyzing the eluate for orthophosphate ion by a spectrophotometric phosphomolybdate method.[20] Typically, an experimental run consisted of making up separate solutions of the β,γ complex and the assisting cobalt amine in 1.0 M tetramethylammonium nitrate. The pH values of the two solutions were adjusted to the desired pH at 40 °C, and equal volumes (usually 1.0 mL) were mixed to start the reaction. The pH of the reaction mixture was maintained by addition of microliter quantities of either 1.0 M nitric acid or 1.0 M potassium hydroxide solution from a microsyringe. At suitable time intervals, 50-μL samples were removed, quenched in 1.0 mL of ice-cold water, and analyzed for orthophosphate. In all kinetic experiments, the β,γ complex concentration was maintained at 25 mM. Kinetics were carried out at 40 °C with the ionic strength held at 1.0 M with tetramethylammonium nitrate. In all cases the rate constant quoted, k_{obsd}, is defined as

$$+\mathrm{d}[\text{total orthophosphate}]/\mathrm{d}t = k_{\mathrm{obsd}}[\beta,\gamma \text{ complex}]$$

NMR Measurements. All ^{31}P NMR spectra were run on a Bruker WP-80 instrument with 85% H$_3$PO$_4$ as an external standard. Spectra were run in aqueous solution containing 30% D$_2$O to provide a signal lock for the instrument. Experiments showed that a delay time of 2 s with a pulse width of 1.6 μs was sufficient to allow complete relaxation of the

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(6) This complex, in which the center phosphate group and one terminal phosphate group are coordinated, is labeled β,γ by analogy with the ATP complex from which crystals for the structure determination were prepared (ref 7). We have also reported the α,β,γ-tridentate binding of triphosphate ion to form two six-membered chelate rings in Co(NH$_3$)$_3$H$_2$P$_3$O$_{10}$. See: ref 7; Merritt, E. A.; Sundaralingam, M. Meeting of the American Crystallographic Association, August 1977; Abstr. F7.

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Table I. Relative Rates of Hydrolysis of Phosphate Species (at pH 6.0, $I = 1.0$ M, and 40 °C)[a]

species	relative rate
$P_3O_{10}{}^{5-}$	1[b]
$Co(NH_3)_4P_3O_{10}{}^{2-}$	7
$Co(NH_3)_4P_3O_{10}{}^{2-} + [Co(tet_a)(H_2O)_2]^{3+}$	7
$Co(NH_3)_4P_3O_{10}{}^{2-} + [Co(Me_6[14]diene)(H_2O)_2]^{3+}$	12
$Co(NH_3)_4P_3O_{10}{}^{2-} + [Co(NH_3)_5(H_2O)]^{3+}$	26
$Co(NH_3)_4P_3O_{10}{}^{2-} + [Co(cyclen)(H_2O)_2]^{3+}$	66
$Co(NH_3)_4P_3O_{10}{}^{2-} + [Co(NH_3)_4(H_2O)_2]^{3+}$	700

[a] The β,γ complex and assisting ammine concentrations were 25.0 and 20.0 mM, respectively. [b] The absolute value under these conditions is 2.3×10^{-7} s^{-1}.

phosphorus nuclei, and these conditions were used for all experiments. Typically 500 pulses were accumulated to generate each spectrum. All pH values for the NMR experiments were recorded directly in the 5-mm NMR tubes by using a Microelectrodes Inc. NMR pH probe. Sample solutions were 0.1 M in EDTA to prevent line broadening by Co(II) species. Spectra were recorded at 302 K.

Results

Product Analysis. A series of experiments was carried out at pH 10.05, 40 °C, and $I = 1.0$ M to determine the nature of the products of the hydrolysis of the triphosphato complex in the presence of cobalt–cyclen species. A 2-mL reaction mixture, 25 mM in both reactants, was allowed to react for about one half-life (~20 min) and was then loaded onto a small (0.5 × 3.0 cm) anion-exchange column. Elution was then accomplished with 0.1 M, pH 8, 1,2-diaminoethane/HCl buffer to remove cobalt–aquo and cobalt–orthophosphato species.[7] The solution coming off of the column was further treated to separate the aquo and orthophosphate cobalt(III) complexes. This separation was carried out on a Sephadex C-25 column (0.5 × 2.0 cm) by elution with aqueous 4 M sodium chloride in 0.05 M nitric acid. Aquo species remained bound to the column and orthophosphate species were eluted. The eluate gave a Co:$PO_4{}^{3-}$ ratio of 0.94, in reasonable agreement with 1.0 ratio expected for cobalt–orthophosphato species. Because the two cobalt(III) atoms in the catalytic solution were chelated by different (N_4) ligands, it proved possible to distinguish orthophosphate ion coordinated to the cobalt(III) atom complexed by cyclen from that coordinated to the ammine complex. This distinction is based on the demonstration that while $[Co(NH_3)_4(H_2O)PO_4]$ is readily reduced by 10% $SnCl_2$ in concentrated HCl, $[Co(cyclen)(H_2O)_2]^{3+}$ is unaffected by this reagent. The orthophosphato complex obtained in the above experiment was not reduced by $SnCl_2$ reagent and was thus shown to be predominantly the cobalt–cyclen–orthophosphato species.

Experiments were also carried out to determine the overall stoichiometry of the reaction. Several 25 mM solutions of the β,γ complex containing Co(cyclen) species were reacted at 40 °C and pH 9.0 for 10 half-lives; the Co(cyclen) species were at 10.0, 20.0, 25.0, and 40.0 mM concentrations. In the final reaction mixtures, concentrations of orthophosphate were found to be 10.0, 20.0, 25.0, and 26.0 mM (±0.1), respectively. These observations are consistent with the stoichiometry

$$[Co(NH_3)_4P_3O_{10}]^{2-} + [Co(cyclen)(OH)_2]^+ \rightarrow [Co(cyclen)(OH)(HPO_4)] + [Co(NH_3)_4(P_2O_7)]^- \quad (2)$$

Table II. pH Dependence of Rate of Hydrolysis of the β,γ Complex in the Absence and Presence of Catalyzing Cobalt–Cyclen Species (at 40 °C and $I = 1.0$ M)[a]

pH	uncatalyzed rate × 10^5/s^{-1}	catalyzed rate × 10^5/s^{-1}
2.00	0.4	3.3
3.00	0.4	4.6
4.00	0.2	3.4
5.00		1.2
6.00	0.5	1.5
7.00	0.1	6.2
8.00	0.3	33.0
8.50		50.0
9.00	~0.3[b]	
9.50		52.6
10.12		54.0
10.60		~30.0[b]

[a] The β,γ complex and assisting cobalt–cyclen concentrations were 25.0 and 20.0 mM, respectively. [b] Decomposition of the β,γ complex occurs over the time interval required for hydrolysis.

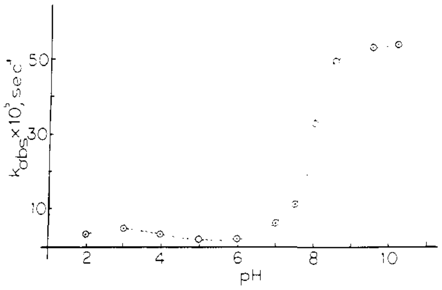

Figure 1. pH Dependence of the rate of hydrolysis of 25 mM β,γ complex in the presence of 20 mM cobalt–cyclen species at 40 °C and $I = 1.0$ M.

Requirements for Rate Acceleration. Earlier studies in this laboratory[8] on the hydrolysis of triphosphate in the β,γ complex, **1**, have examined the hydrolysis in the presence of other cobalt species possessing at least one "vacant" site; a "vacant" site being one occupied by a labile ligand with which the reacting species can readily interchange. For maximum effect, two "vacant" sites which are cis to one another appear to be a requirement. As can be seen from Table I, which reports the effects of a variety of cobalt(III) amine complexes on the rate of hydrolysis of the β,γ complex, the only amine complexes which show any significant effect are the compounds $[Co(NH_3)_4(H_2O)_2]^{3+}$ and *cis*-$[Co(cyclen)(H_2O_2]^{3+}$. The two compounds with trans diaquo ligands, the cobalt complexes of $Me_6[14]$diene and tet_a, show much smaller accelerating effects, and this observation becomes important during the discussion of possible mechanisms for the cobalt(III)-assisted phosphate hydrolysis.

pH Profile. Hydrolysis rates for the reaction of the β,γ complex both in the presence and absence of the complex *cis*-$[Co(cyclen)(H_2O)_2]^{3+}$ are given in Table II for experiments in which the concentration of the β,γ complex is 25 mM and the cyclen species 20 mM. The results in Table II for the experiments in the absence of the cyclen complex over the pH range 2–9 show little dependence upon pH. These results contrast with those for free triphosphate ion, for which the rate of hydrolysis increases with acid concentration at low (<7) pH and a constant rate is achieved at higher hydroxide ion concentrations.[21] The rate constant for hydrolysis of free triphosphate ion in the pH range 8–10 has been reported to be ~8 × 10^{-8} s^{-1} at 60 °C,[21] in agreement with the value at 40 °C, 1.0 M ionic strength, and pH 9, determined in this research to be 1.86 × 10^{-8} s^{-1}. A comparison of this value with the results in Table II reveals that at pH 9 the β,γ complex enhances the rate of triphosphate hydrolysis by a factor of about 160, although this enhancement at pH 6 is only of the order of 7. The effect of adding the cobalt(III) cyclen complex is even more marked. Figure 1 shows that for the series of experiments in the presence of the cyclen complex there is again only a slight dependence on pH below pH 7, but at higher values a marked increase is observed and the rate constant rises rapidly to a plateau level at pH ~9.5. The curve in Figure 1 closely resembles a titration curve for a weak acid, and if the assumption is made that the catalytically active complex is the conjugate base of some protonated species in the reaction mixture, a value for the p*K* of this species can be calculated. If the rate of hydrolysis, corrected for the background rate of the free β,γ complex, is considered to be directly proportional to the concentration of

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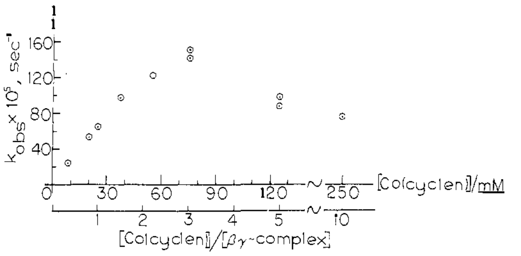

Figure 2. Dependence of the rate of hydrolysis of 25 mM β,γ complex on the concentration of cobalt–cyclen species at pH 10.05, 40 °C, and I = 1.0 M.

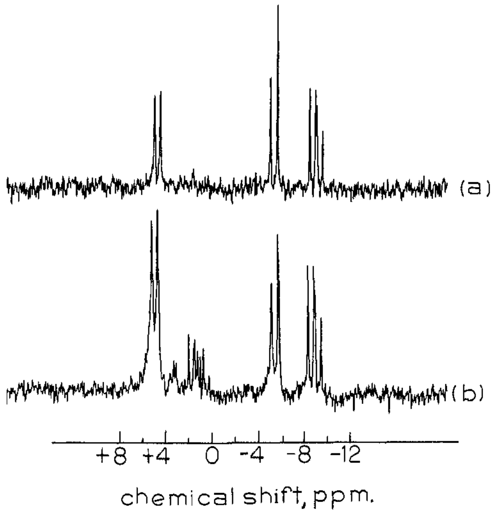

Figure 3. ^{31}P NMR spectrum at 32.4 MHz of the β,γ complex in the absence (a) and presence (b) of cobalt–cyclen species at pH 6.0.

conjugate base in the system, then the pK of the species can be calculated by application of the Henderson–Hasselbach equation:[22]

$$pK' = pH - \log([B^-]/[BH]) \quad (3)$$

In this equation pK' is a practical pK of the species, B^- is the concentration of the conjugate base and BH is the concentration of protonated species. Calculation using several points on the pH profile curve gives a pK' for the specis of 7.9 ± 0.3 at 40 °C and ionic strength 1.0 M.

Attempts to measure the hydrolysis rates of the free β,γ complex at these high "plateau" pH values proved unsuccessful due to rapid decomposition of the β,γ complex, which was readily detected by performing the reactions under a stream of nitrogen gas and then testing for ammonia in the effluent gas with Nessler's reagent.[23] This difficulty was not encountered with the cyclen complex assisted reactions as the rate of hydrolysis in this latter case was sufficiently high to enable orthophosphate production to be monitored before significant decomposition had occurred.

Concentration Dependence. The effect of changing the relative concentrations of the β,γ complex and the cyclen complex is shown in Figure 2. At low ratios of the β,γ complex to cyclen concentration, the plot of k_{obsd} vs. cyclen complex concentration is linear with a slope of unity. As the ratio is increased, however, the rate begins to show less than a first-order dependence on the cyclen complex concentration and indeed appears to fall off at high ratios (>3) of [Co(cyclen)]/[β,γ complex]. A maximum rate is reached at a cyclen complex to β,γ complex ratio of about 3:1, and at those relative concentrations the observed rate of hydrolysis is 1.4×10^{-3} s^{-1}, a rate enhancement of 460 over the rate for the β,γ complex alone and 75 000 over that for free triphosphate ion under the same conditions.

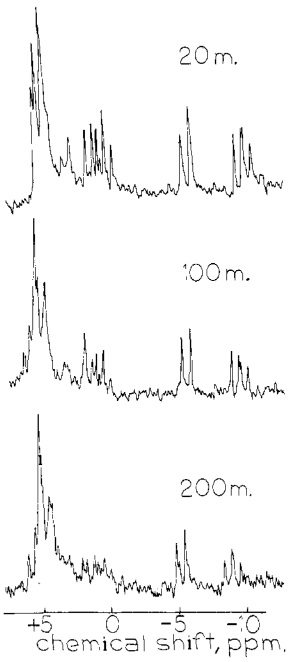

Figure 4. Change in ^{31}P NMR spectrum at 32.4 MHz at the β,γ complex in the presence of cobalt–cyclen species at pH 7.8 over time intervals of 20, 100, and 200 min at 40 °C.

^{31}P NMR. Results of the pH profile experiments and concentration-dependence measurements indicate that there is some complexation between the β,γ complex and the cobalt–cyclen complex. The most likely interaction would be between the uncoordinated, terminal α-phosphate group and one of the two labile cis sites on the cyclen complex.

Two sets of ^{31}P NMR data were recorded. Those shown in Figure 3 were recorded at pH 6.0 with a β,γ complex concentration of 0.1 M. The spectra in the absence and in the presence of 0.1 M $[Co(cyclen)(OH)(H_2O)]^{2+}$ are shown (Figure 3, a and b, respectively). At this pH, hydrolysis is slow enough to enable collection of sufficient transients to produce a spectrum before hydrolysis products appear. As can be seen, in the presence of the cyclen species, a whole new series of phosphorus resonances appear in the range 0–2 ppm.

The spectra in Figure 4 correspond to those of a similar system containing 0.1 M β,γ complex and cyclen species maintained at pH 7.8. The three spectra show the time dependence of the ^{31}P NMR spectrum, the three spectra being recorded at 20, 100, and 200 min after the start of the reaction. The times given are only approximate and refer to the time at which data acquisition was started for each spectrum. At pH 7.8, considerable hydrolysis occurs over the 10-min period required for the accumulation of data for each spectrum.

Analysis of the relative areas under the peaks associated with α-, β-, and γ-phosphate groups, assigned by using previously published data[7] (doublet at −5.25, double doublet at −8.96, and a doublet at +4.24 ppm, respectively), gave the expected 1:1:1 ratio for the free β,γ complex. However, in the presence of the cyclen complex the ratios change to 0.6:0.6:1 at pH 6 and 0.4:0.4:1 at pH 7.8 for the α-, β-, and γ-phosphates. This change lends support to the argument that at least some of the species in solution are coordinated through the α- and/or β-phosphate groups, and furthermore, the extent of the coordination is pH dependent. Several possibilities could exist and are identified in the Discussion. The ^{31}P NMR spectra in Figure 4 also demonstrate the appearance of one of the predicted reaction products, that is, bound pyrophosphate, which appears initially as a shoulder on the downfield side of the γ-phosphate resonance and gradually grows to dominate the spectrum in this region (5.1 ppm).

Titration Data. The complex *cis*-$[Co(cyclen)(H_2O)_2]$ was generated by means of an anion column in the hydroxide ion form as described earlier in this work, and 5×10^{-4} mol samples of the

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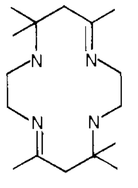
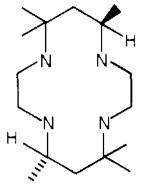
diaquo species were titrated with 0.1 M potassium hydroxide at 40 °C and $I = 1.0$ M to determine the pK values of the coordinated water molecules. Treatment of the titration results by the Noyes method[24] gave values of 5.11 (±0.05) and 7.54 (±0.05) for these two pKs.

Discussion

Previous work in this laboratory has demonstrated an accelerating effect when labile positions are available on the added cobalt(III) species.[8] The most marked effects so far studied are observed when two sites, cis to one another, are available. This observation has been confirmed by this work, where use of a 100% cis diaquo species and two 100% trans diaquo species have born out the previous results. The cyclen species is 100% cis by virtue of the fact that the "hole size" in the center of the macrocycle is too small to accept a cobalt atom in the plane of the four nitrogen donor atoms, so the ligand must fold slightly to accommodate the metal atom.[10] The two other macrocyclic ligands used were a saturated 14-membered ring and an unsaturated 14-membered ring system. The unsaturated macrocycle Me$_6$[14]-diene (**3**) yields a trans diaquo species when complexes with cobalt(III) because folding is hindered by the planar imine linkages in the ring of the macrocycle. Similarly, the saturated ligand tet$_a$ (**4**) gives a trans diaquo system. In the case of tet$_a$, folding is prevented by steric hindrance of the two *gem*-dimethyl groups.[18]



3



4

The results shown in Table II for the hydrolysis of the β,γ complex in the absence of any other cobalt complex show very little dependence on pH and give a rate constant for hydrolysis of the free β,γ complex of 4×10^{-6} s^{-1} at 40 °C, which is at least 7 times larger than that for the free triphosphate ion at pH 6. At higher pH (8.0–10.5), the rate of hydrolysis of triphosphate is much slower,[21] and thus the catalytic effect of the β,γ complex on triphosphate hydrolysis is much greater at pH 9 than at pH 6. In fact, the free β,γ complex hydrolyzes some 200 times faster than free triphosphate ion at pH 9. In the complex-promoted group of experiments, there is very little dependence on pH at pH <7, the rate enhancement being fairly constant and of the order of 10 over the free β,γ complex or 70 over free triphosphate ion.

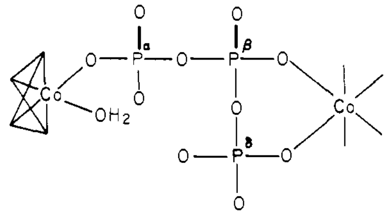
At pH values greater than 7, however, the catalyzed series of experiments start to show a very marked pH dependence, and the rate of phosphate hydrolysis increases dramatically. As a result of this very much enhanced hydrolysis rate at high pH values, it was possible to measure the rate of hydrolysis of the β,γ complex before significant reduction of the β,γ complex occurred, enabling a "plateau" region to be detected in the pH profile at very high pH (>9.5).

The very rapid increase in the rate on changing the pH of the reaction solution from 7 to 9 indicates that the active species has a pK of 7.9. Several ionizable species exist in the reaction solution, but those on the β,γ complex phosphate groups are readily ruled out on the basis of their known pK values. There are two available protons on the phosphate groups of the β,γ complex, the pKs of which have been determined to be 2.2 and 5.7,[1] both of which are too low to meet the value of 7.9 required by our experiments. Furthermore, if deprotonation of a phosphate group was necessary to produce the catalytic species, it would be difficult to rationalize the comparative inability of the pentaammine–aquo species or the trans diaquomacrocyclic complexes to catalyze the reaction. Amine hydrogen atoms on the macrocyclic ligand are another potential source of ionizable protons, but again these are ruled out as their pK values are in a much higher range, usually greater than 14.[25,26] The third remaining source of an ionizable proton in the catalytic system would be the coordinated water molecule in the proposed intermediate complex shown in structure 5.

Table III. pK Values of Water Molecules Coordinated to Cobalt(III)

species	pK_1	pK_2	ref
$[Co(NH_3)_5(H_2O)]^{3+}$	6.21		32[a]
$[Co(NH_3)_4(H_2O)_2]^{3+}$	5.69	7.99	33[b]
$[Co(CN)_5(H_2O)]^{2-}$	9.70		28[c]
$[Co(en)_2(H_2O)HPO_4]^{+}$	6.75		27[e]
cis-$[Co(cyclen)(H_2O_2]^{3+}$	5.11	7.54	this work[c]
trans-$[Co(tet_a)(H_2O)_2]^{3+}$	2.70	6.8	34[d]
trans-$[Co(Me_6[14]diene)(H_2O)_2]^{3+}$	4.02	8.4	34[d]

[a] $I = 0.3$ M, 25 °C. [b] $I = 0.1$ M, 20 °C. [c] $I = 1.0$ M, 40 °C. [d] $I = 1.0$ M, 25 °C. [e] $I = 1.0$ M, 22.5 °C.



5

A pK value of 7.9 is a reasonable value for a water molecule coordinated to cobalt(III). Under the conditions of the experiment, the pK of the coordinated water molecule in structure **5** could be realistically compared to the pK_2 values of cis diaquo species as the environment of the coordinated water in the intermediate, **5**, is cis to a negatively charged phosphate group. This situation is analogous to the second water molecule in a cis diaquo system after the first coordinated water molecule has been ionized; the remaining water molecule would be cis to a negatively charged group, in this case a hydroxide ion. Consulting Table III reveals that pK_2 values in the range of 7–8 are quite reasonable and give credence to our assignment of the pK of 7.9 to the coordinated water molecule in structure **5**.

The most plausible mechanism invoking the postulated coordinated hydroxide ion cis to the phosphate group would be internal nucleophilic attack by the coordinated hydroxide ion on one of the phosphate residues. This type of internal nucleophilic attack in cobalt(III) amine complexes has been postulated and fairly well established in a mechanism in cobalt(III)-assisted hydrolysis of amino acid esters, amino acid amides, and dipeptides.[28,29] Indeed, there is also evidence for internal attack by coordinated NH_2 on amino acid esters[30] and phosphate esters[31] so the effect is not just limited to hydroxide ion. Experiments are in progress to determine whether other coordinated nucleophiles will catalyze hydrolysis of coordinated triphosphate ion.

The existence of a dinuclear cobalt speices of the type suggested is supported by the appearance of new phosphorus resonances in

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the ^{31}P NMR spectra of the reaction mixtures at both pH 6 and 7.8. That these new resonances in the 0–2 ppm range are not due to product formation can be shown in Figure 4, as these peaks disappear with time. The intermediate complex **5** possesses five optically active centers—the β-phosphorus atom and the four secondary nitrogens of the macrocyclic ligand—and numerous diastereomers that would be potentially distinguishable by NMR could exist. Thus the interpretations of any new phosphorus resonances in the intermediate (**5**) at 0–2 ppm is greatly complicated by stereochemical considerations and falls outside the scope of this paper.

The exact phosphorus group that is attacked has not yet been determined. Dreiding models of the intermediate (**5**) suggest that the β-phosphate is sterically the most favored, though the work of Lincoln and Stranks[27] suggests that at pH 9 bidentate orthophosphate or a four-membered chelate ring is quite stable, so attack at the α-phosphate is not ruled out. In any case, attack at the γ-phosphate seems unlikely, since as has already been pointed out, orthophosphate from the hydrolysis reaction remains bound to the cyclen species. The stoichiometry experiments show that under the conditions used for these experiments only one molecule of orthophosphate is generated by each molecule of complex 5; thus orthophosphate production is limited by either the β,γ complex concentration or the Co(cyclen) species concentration, whichever is the smaller. Complete hydrolysis of triphosphate to three molecules of orthophosphate can occur if sufficient time is allowed, but over the time scale of the cobalt(III)-assisted reaction these subsequent reactions may be neglected. On basis of the stoichiometry, the pH dependence of the rate, and the NMR species, the following seems a likely reaction scheme:

$$\beta,\gamma \text{ complex } + \text{[Co(cyclen)(OH)}_2]^+ \overset{K_e}{\rightleftharpoons} \text{deprotonated complex } \mathbf{5} \qquad (4)$$

$$\text{deprotonated complex } \mathbf{5} \xrightarrow{k_r} \text{products} \qquad (5)$$

where K_e is an equilibrium constant and k_r is a first-order rate constant for dissociation of the intermediate into products.

Having postulated the existence of a structure of the type suggested in **5**, it becomes apparent from the results of the concentration-dependence experiments that an equilibrium must exist between the catalytic complex **5** (eq 3) and the free β,γ and cyclen complexes, since a 1:1 ratio of these latter two compounds does not give the maximum rate enhancement. An equilibrium of the form in eq 4 could apply.

The following rate law can be derived for the above model:

$$k_{obsd} = (k_r K_e[C])/(1 + K_e[C]) \qquad (6)$$

where [C] is the concentration of cyclen complex and k_{obsd} is the observed rate constant. Equation 5 can be rearrange to give

$$1/k_{obsd} = 1/(k_r K_e[C]) + 1/k_r \qquad (7)$$

Thus, by use of the experimentally determined parameters k_{obsd} and [C], values for k_r and K_e can in principle be determined. If the first five linear points in Figure 2 are used in a reciprocal plot as is required by eq 6, a straight line of intercept $1/k_r$ and slope $1/k_r K_e$ is obtained. In practice, large uncertainty in k_r results due to the fact that, as with most reciprocal plots, a small intercept value on a very large ordinate scale is being computed. Even so, an approximate value for K_e was determined to be 3.0 (±1.5) M^{-1}, and k_r came out to be 0.010 (±0.006) s^{-1}. Even with the inaccuracies in measuring k_r, the rate enhancement over the rate of hydrolysis of free β,γ complex is very marked at high pH, being of the order of 3×10^4. The enhancement over the rate of hydrolysis of free tripolyphosphate at high pH is of course even more marked and comes out to be about 5×10^5. Although these numbers are subject to uncertainty, the enormous effect of this particular macrocyclic amine complex on the hydrolysis rate of triphosphate in aqueous solution has been effectively demonstrated.

The fact that at high cyclen concentrations the rate of hydrolysis of the β,γ complex appears to slow down (Figure 2) is difficult to rationalize at present. A greater knowledge of the nature of the catalytic intermediate (structure **5**) is required before the mechanism of this apparent inhibition could be postulated. In any event, it seems likely that a tri- or even tetranuclear cobalt(III) species may be involved.

In conclusion the results of this research have shown that cobalt ammines have the ability to greatly enhance the rate of triphosphate hydrolysis, by factors as large as 10^5. Maximum effects appear to be brought about by dinuclear cobalt species that function as templates, positioning the triphosphate ion and incoming nucleophile to facilitate the reaction.

Acknowledgment. We are grateful to Professor Grover Everett of the University of Kansas for provding access to NMR instrumentation. This research was supported by Research Corporation and National Science Foundation Grant No. CHE-8024043.

Registry No. $P_3O_{10}^{5-}$, 14127-68-5; $Co(NH_3)_4P_3O_{10}^{2-}$, 63915-32-2; *trans*-$[Co(teta)(H_2O)_2]^{3+}$, 39828-60-9; *trans*-$[Co(Me_6[14]diene)(H_2O)_2]^{3+}$, 17815-30-4; $[Co(NH_3)_5(H_2O)]^{3+}$, 14403-82-8; *cis*-$[Co(cyclen)(H_2O)_2]^{3+}$, 81064-04-2; $[Co(NH_3)_4(H_2O)_2]^{3+}$, 14877-14-6.

Dealkylation of Zirconium(IV) by Borane: The Intimate Mechanism of an Alkyl Transfer Reaction

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Abstract: The reaction of Cp_2ZrMe_2 with $BH_3 \cdot THF$ ultimately yields $Cp_2Zr(BH_4)_2$. The reaction is sequential in the sense that $Cp_2Zr(BH_4)Me$ has also been detected. By use of ^{11}B NMR spectroscopy, both for resolution of chemically distinct boron-containing species and as an aid to proton counting, it has been possible to show that these reactions proceed through intermediates containing coordinated BH_3Me^- and $BH_2Me_2^-$, and thus the reaction is initially a formal insertion of BH_3 into a Zr–CH_3 bond. These intermediates react further with borane to produce a complex equilibrating mixture of alkyldiboranes, $B_2Me_nH_{6-n}$, and $Zr(BH_4)$ groups.

In the course of our investigation of the transfer of hydrogen from transition-metal hydrides to the η^2-acetyl group in $Cp_2Zr[C(O)Me]Me$,[1] it became important to establish the reactivity of a neutral main group hydride toward this same acetyl. Bo-

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