Coordination Chemistry Reviews, 79 (1987) 293–319
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

HYDROLYSIS OF PHOSPHATE ESTERS AND ANHYDRIDES: ROLE OF METAL IONS

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(Received 1 August 1986)

CONTENTS

A. INTRODUCTION

In biological systems the role of phosphoryl (PO_3^-) transfer reactions in the storage and utilization of energy, as in the formation and hydrolysis of ATP, is well known [1]. Another well known feature of bio-phosphate chemistry is the structural stability of phosphate ($RO{-}PO_2{-}OR$) and pyrophosphate ($R{-}O{-}PO_2{-}O{-}PO_2{-}O{-}R$) diesters in the backbones and links of complementary strands of DNA. PO_3^- transfer reactions involve rupture of normally very robust P(V)–O bonds, as evidenced by the inertia of polyphosphates (ATP) and phosphate esters to uncatalyzed hydrolysis. Thus, the ability of enzymes, literally, to direct such processes (like the hydrolysis of ATP) to take place when the system needs energy and not allow the hydrolysis of structural phosphate esters to occur in the same system, creates an intriguing mechanistic problem. The fact that metal ions, usually Mg^{2+}, participate in the enzymic processes has led to considerable research into the hydrolysis of polyphosphates and phosphate esters as catalyzed by or effected by metal ions [2]. Our group has had a modest interest in such reactions for about 14 years since Scott and co-workers discovered that oxidizing VO^{2+} complexes of PPP_i and ATP produces a 1000 fold increase in the hydrolysis rates induced by VO^{2+} [3]. We have also, in collaboration with Sargeson's group in Canberra, investigated hydrolyses effected by complexes formed of $N_4Co(OH)(OH_2)^{2+}$ complexes with polyphosphates [4,5]. The latter can be studied using ^{31}P NMR which gives structural information on precursor complexes, intermediates, and products. We can now compare the behavior of labile and inert metal ions, and structural and kinetic factors promoting and inhibiting phosphoryl transfer processes.

In all cases, results indicate that the metal ion effecting hydrolysis is bound monodentate to a terminal–PO_3^- group with OH^- or O^{2-} ligated in a *cis* position poised for nucleophilic attack on a P(V) center. Two active complexes which we have postulated and previously reported are shown in Fig. 1 [3,4]. One labile VO^{3+} can coordinate and effect scission of terminal PO_3^- from linear triphosphate [3] while two tn_2Co^{3+} must bind to pyrophosphate already chelated to en_2Co^{3+} and, with concerted nucleophilic attack on one P(V) center and electrophilic attack on the bridging oxygen, effect scission of the most robust P–O–P bond known [5]. Here we see Co(III) atoms performing all the functions required of the active center in pyrophosphatase (described by Dunaway-Mariano and co-workers [6] (Fig. 2)) where pyrophosphate chelated to one Mg^{2+} is bound in a pocket designed to provide for nucleophilic attack on P(V) at one end of the pyrophosphate

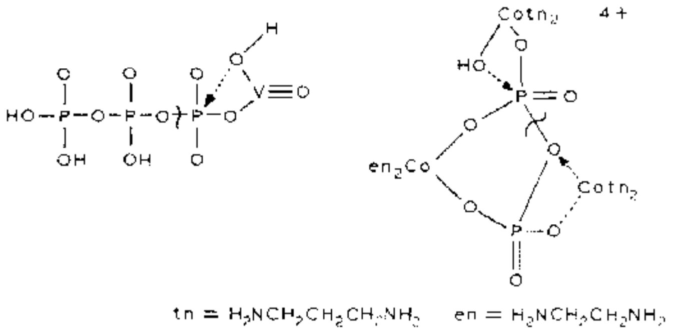


Fig. 1. Representative activated states for metal assisted hydrolysis of triphosphate [3] and pyrophosphate [4].

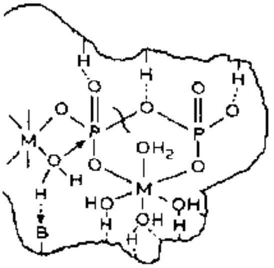


Fig. 2. Schematic model for the active site in pyrophosphatase [6]. $Mg(H_2O)_4PP_i$ is hydrogen bonded to the enzyme. A second Mg^{2+} may be positioned for nucleophilic attack by OH^-. This figure is modified from ref. 2 by suggestions of in-line attack of OH^- on a P(V) center opposite the P–O bridge to be broken, and that the second metal ion is bound monodentate to the pyrophosphate.

(e.g. OH^- bound to a second Mg^{2+}) and electrophilic attack (e.g. by H^+) at the other end or on the bridging O atom (Fig. 2).

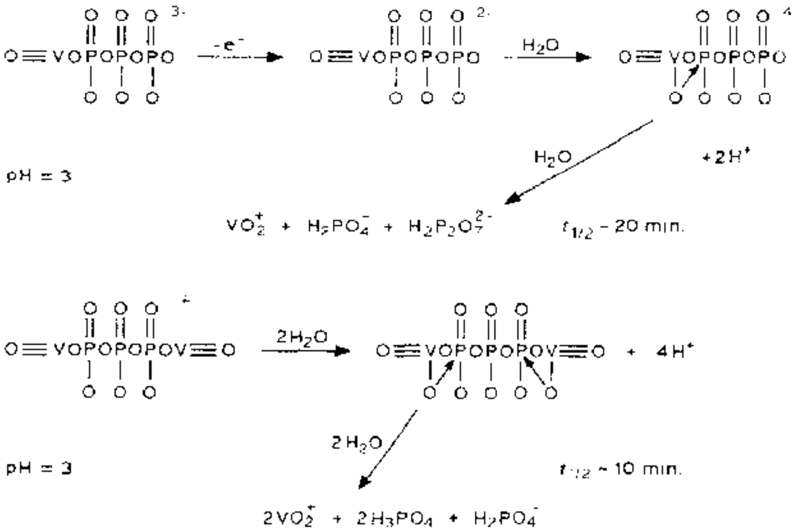
Similarly, an active enzyme site for $ATP–H_2O \rightarrow AMP + PP_i$ has been characterized by Fersht [7] as having pockets in which the adenosine, sugar, and triphosphate portions would each fit and bind by non-covalent interactions to the protein. The nucleophile in this case could well not be OH^- ligated to bound metal ion, unless the metal ion itself were part of the template in position to attack the α-phosphorus. In vitro in aqueous solution at pH 7, we find that metal ions (Co(III)) with coordinated OH^- bind to the more basic γ-phosphorus of ATP leading to hydrolysis of the terminal PO_3^- to form ADP and P_i.

With these postulated models for enzymic scission of P–O–P bonds in pyrophosphate and triphosphate for reference, this paper will report on recent and current results of the hydrolysis of various pyrophosphates and triphosphates complexed with $V{\equiv}O^{3+}$ and tn_2Co^{3+} giving relative rates and structures deduced for active complexes and activated states like the examples given in Fig. 1. Much of this will be work in progress or in the process of being written for subsequent publications on the individual systems described.

B. HYDROLYSIS OF TRIPHOSPHATE AND PYROPHOSPHATE BY LABILE $V{\equiv}O^{3+}$

Hydrolysis of triphosphate [3] following oxidation of V(IV) complexes, $[VO(P_3O_{10})]^{3-}$ and $[(VO)_2(P_3O_{10})]^-$, is shown in Scheme 1. $VO(P_3O_{10})_2^{7-}$ does not hydrolyze. V(V) bridging two triphosphate ions cannot attack either one with a coordinated O^{2-} or OH^-.

All hydrolyses follow first order kinetics, which is best interpreted if one assumes complete formation of the complexes in Scheme 1 in 2 : 1, 1 : 1, and



Scheme 1

1 : 2 VO/P_3O_{10} ratios at 0.005 M triphosphate, pH 3 and $T = 25°C$. V(V) complexes do not affect the ^{31}P NMR signals, except to broaden the lines and remove the fine structure. If the postulated complexes are very labile, then such a result with NMR is possible. All conclusions we have been able to make on V(V) induced P–O–P bond scission and the structure of activated states has had to be inferred from kinetics and stoichiometry using chemical intuition [3].

(i) Hydrolysis of pyrophosphate

Recent results (Fig. 3 and 4) show a very different pattern for V(V) induced hydrolysis of pyrophosphate [8]. 1 : 1 Solutions hydrolyze so slowly that no attempt was made to follow them to completion during early studies of the oxidized vanadium system [3]. A recent attempt to follow the hydrolysis with ^{31}P NMR over about two weeks, projected as necessary for the completion of the reaction, revealed a pattern of auto-acceleration (Fig. 3). Reactions accelerated until a large fraction of the $P_2O_7^{4-}$ had hydrolyzed and then became first order with a common half-life. Increasing the $V(V)/P_2O_7^{4-}$ ratio increased initial rates until at a ratio of 4 : 1 the reaction became first order initially, with the same half time as that observed at the end of the autoaccelerated reactions. This suggests that the reactive complex contains four V(V) species attached monodentate to the four basic oxygens on the $P_2O_7^{4-}$ ion and hydrolyzes according to Scheme 2.

Hydrolyses of oxidized complexes of V(IV) are much faster than of complexes formed by mixing solutions of VO_2^+ with polyphosphates in

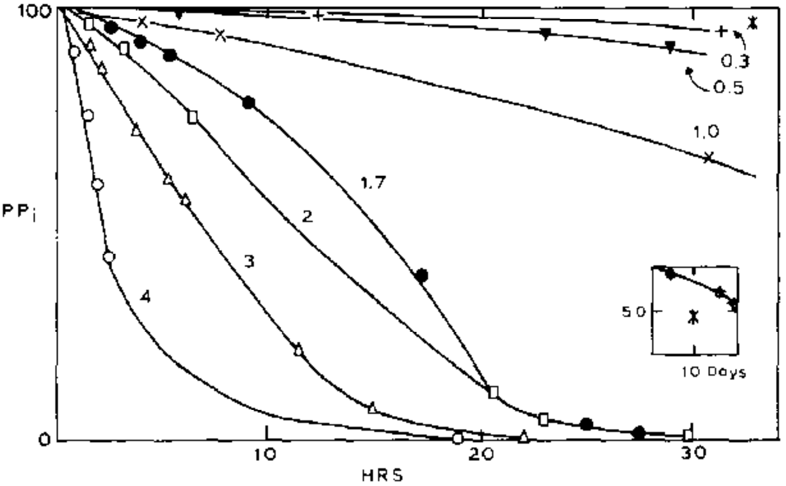


Fig. 3. Hydrolysis of 0.1 M PP_i at $pH = 1$. Auto-accelerated hydrolyses of pyrophosphate induced by oxidation of $(VO^{2+})_nPP_i$ complexes. The value of n for each run is indicated by each curve. First order hydrolysis occurs when $n \geqslant 4$.

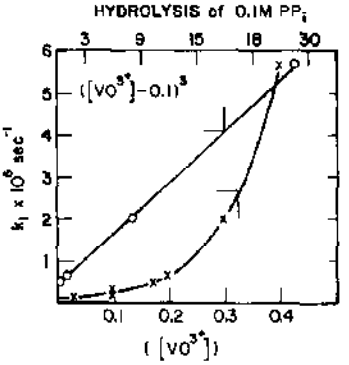
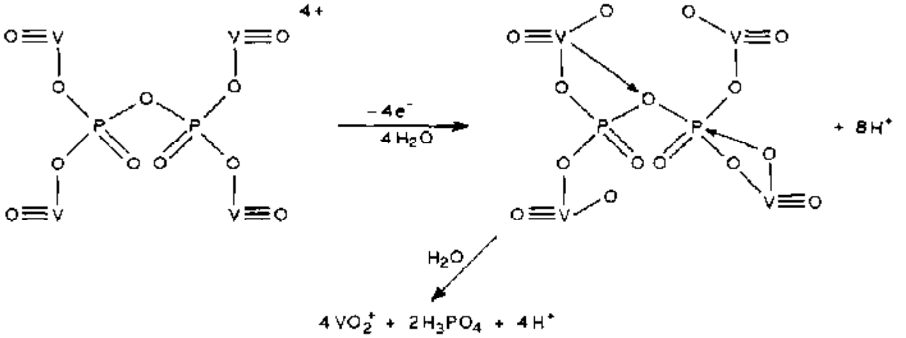


Fig. 4. A plot of constants for initial rates in Fig. 3, vs. concentration of oxidized vanadium present. The linearity of the plot of the cube of excess VO^{3+} over that needed to form a 1 : 1 complex suggests that $(VO^{3+})_4PP_i$ is the complex which hydrolyzes.



Scheme 2. With all four basic oxygen atoms bound to VO_2^+ the vanadium cannot chelate by substitution but can only induce nucleophilic attack on P(V) centers and assist hydrolysis by electrophilic attack on the bridging oxygen. The long (~ 2 h) half time for this process indicates the low probability for this activated state to occur.

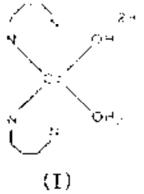
which case equilibrium with incomplete complex formation is established. The higher charge on VO^{2+} permits complex formation to be complete, initially.

C. STUDIES USING THE Co(III) COMPLEX, $tn_2Co(OH)(OH_2)^{2+}$ (I)

Hydrolyses of polyphosphates are effected by substitutionally inert *cis*-$N_4Co(OH)(OH_2)^{2+}$ complexes [4,5,9–17]. Hydrolyses of ATP and ADP are each effected by two moles of $tn_2Co(OH)(OH_2)^{2+}$ per mole of polyphosphate [15,17] while that of linear triphosphate ($P_3O_{10}^{5-}$) is enhanced by 7.5×10^4 relative to free triphosphate by three moles of cyclen-$Co(OH)(OH_2)^{2+}$ [10]. Many 1 : 1 chelated complexes have been isolated for similar $N_4Co(III)$ polyphosphates which show little, if any, enhancement of hydrolysis. Chelation of the inert Co(III) atom prevents nucleophilic attack on P(V) centers; binuclear and trinuclear Co(III) complexes limit one or two Co(III) centers to monodentate binding to phosphates and thus are able to effect hydrolysis. Our group has been studying reactions of $tn_2Co(OH)(OH_2)^{2+}$ (complex formation and hydrolysis) with phosphates (esters and complexes) which contain terminal PO_3^- groups with only one basic oxygen, or are adjacent to a PO_4 group with no basic oxygen to which Co(III) might chelate. Chemical shifts due to bound Co(III) are useful (Table 1) in identifying reactants, products, and intermediates over time as complexes form and decay and hydrolyses occur [4,5].

(i) Properties of $tn_2Co(OH)(OH_2)^{2+}$ (I)

This ion (I) undergoes rapid exchange with H_2O (~ 1 s) and so forms monodentate complexes fast with phosphates in aqueous solution. The OH^- does not exchange rapidly. In neutral solution it will chelate with ortho, pyro, and triphosphate or form a bridge between two phosphates giving a rise in pH due to the loss of the OH^- ligand. The ion was usually available in the *trans* form as the perchlorate, but is nearly 100% *cis* in aqueous solution. It can eliminate water forming a *cis*-μ,μ'-dihydroxy bridged dimer. Under the conditions of our studies it is liable to reduction by water giving paramagnetic Co(III) species which spoil NMR signals.

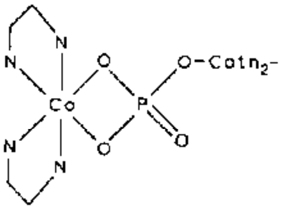
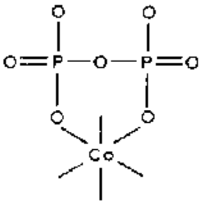
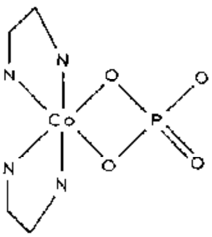


(I)

Experiments are initiated by adding solid *trans*-$tn_2Co(OH)(OH_2)(ClO_4)_2$ to solutions of the phosphate complexes to be studied. Monodentate com-

TABLE 1

^{31}P Chemical shifts for typical phosphates; complexes and structures

Complex	∂ vs H_3PO_4/ (ppm)	Complex	∂ vs H_3PO_4/ (ppm)
PO_4^{3-}	+6		
$P_2O_7^{4-}$	−6		+31 to 35
$P_3O_{10}^{5-}$	−6, −19		+4
	+24		

Add Co(III) monodentate (+6 to 8 ppm): bidentate (+10 ppm); add Co(III) bidentate (+10 ppm for each bond); add PO_3^- (−12 ppm); add H^+ (−1 to −3 ppm).

plexes form in seconds assisting the solution process and preventing dimerization of *cis*-(**I**). Table 1 shows some typical ∂ values versus H_3PO_4 for some typical phosphates, complexes, and structures, and the additive ∂ values to be applied for different atoms and groups found bound to PO_4^{3-} in these systems [4,5,9–12,14,15].

(ii) *Reactions of* $tn_2Co(OH)(OH_2)^{2+}$ *with terminal* PO_3^- *groups having one basic oxygen atom*

(a) $N_4Co(PO_4)$ *chelates*

Figure 5 shows the sequence of NMR signals observed during the overall reaction shown in Scheme 2 [13]. The sequence is essentially the same regardless of whether the four nitrogen atoms on the initial Co(III) chelated orthophosphate are supplied by 2 en, 2 tn, or 4 NH_3 ligands.

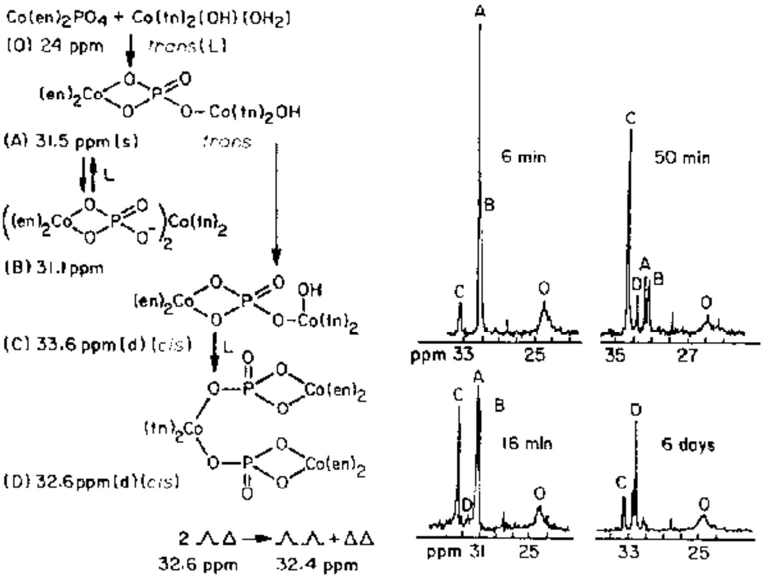
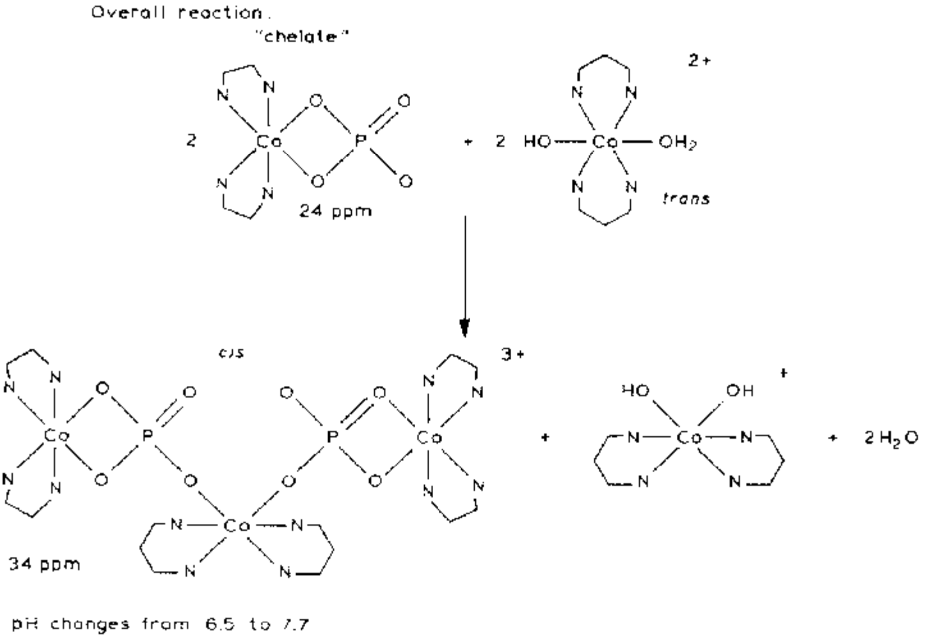


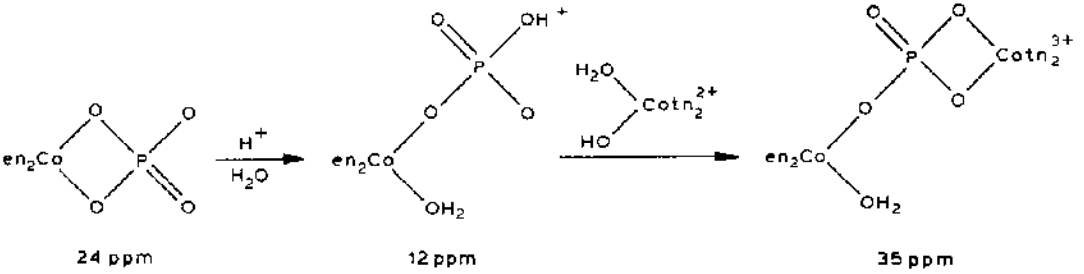
Fig. 5. ^{31}P NMR signals vs. time for the reaction 0.10 M en$_2$CoPO$_4$ chelate (∂ = 24 ppm) with 0.1 M tn$_2$Co(OH(OH$_2$)$^{2+}$ in unbuffered solution: pH rises from 6.5 to 7.7 (Scheme 3).

^{18}O Exchange experiments indicate that no P–O bond breaking occurs in this system [13]. Thus, only complex formation of (I) with a monobasic terminal PO$_3^-$ takes place. This involves four principal steps: (1) Monoden-



tate binding of *trans*-(I) to the basic oxygen of the chelated orthophosphate (^{31}P singlet at 32 ppm.) (2) Substitution of a second chelate for the OH on (I) forming a *trans* bridging complex between two chiral chelates, raising the pH (^{31}P doublet at 31 ppm). (Presumably the binding of Co(III) to two phosphates slightly towers the ∂ values for monodentate Co(III) on the P(V) NMR signal.) (3) *Trans* complexes become *cis* in the polar solvent, at first accompanied by dissociation of one chelate from the *trans* bridged complex to give a *cis* complex of (I) bound monodentate to chelate (^{31}P doublet at 35 ppm). (4) A *cis*-Co(III) bridged complex forms in which all three Co(III) centers are chiral (^{31}P doublet at 34 ppm). One half of the doublet appears to change to the other half, becoming a singlet in about a week's time. These signals at 31–35 ppm represent the probable signals to be found when a PO_3^- group is hydrolyzed by action of bound (I) analogous to the V(V) action described in Fig. 1. The system appears to approach equilibrium with most of the product phosphate present as the product of step (4), but with each of the other species present in small amounts. The NMR signal of the starting chelate is broadened indicating that some labile monodentate complex is formed with (I) as well as inert complexes giving the well defined chemical shifts.

In acid solution, the chelate ring opens before (I) is added. The first, and principal, reaction with (I) is



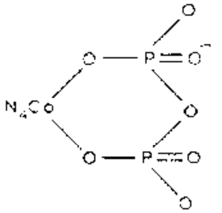
giving the product of step 3 in Scheme 3 [13].

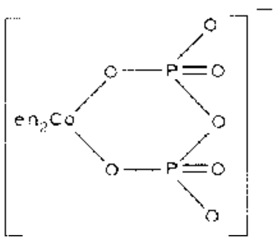
$(NH_3)_5CoOPO_3$, which has two basic O atoms on the PO_3^- group, gives mixed products with (I); some chelate (33 ppm) and some Co(III) bridges between two molecules of $(NH_3)_5CoOPO_3$ (16–18 ppm) [9].

Preliminary trials indicate that AMP also gives primarily a monodentate complex with (I) at pH values from 4–10. Cyclic AMP gave no reaction with (I) in neutral solution, the signal for cyclic AMP being unperturbed by the addition of (I). Evidently the one basic oxygen on this diester is too weak to bind Co(III) in (I). $H_2PO_4^-$ and HPO_4^{2-} react incompletely with (I) giving a mixture of bridged complexes and the chelate described above. The latter can be isolated, crystallized and used for studies like that in Scheme 3.

(iii) Reactions of (I) with complex pyrophosphates with one basic oxygen on one or both terminal PO_3^- groups, A

Essentially equivalent results are obtained when the four nitrogen atoms are supplied by 2 en, 2 tn, or 4 NH_3 molecules. The stable six-membered ring forms rapidly and quantitatively at pH 7 in 1 : 1 solutions of labile (for water exchange) $tn_2Co(OH)(OH_2)^{2+}$ (I) and $P_2O_7^{4-}$ to give inert $tn_2CoP_2O_7$ which is easily isolated as the uncharged acid.





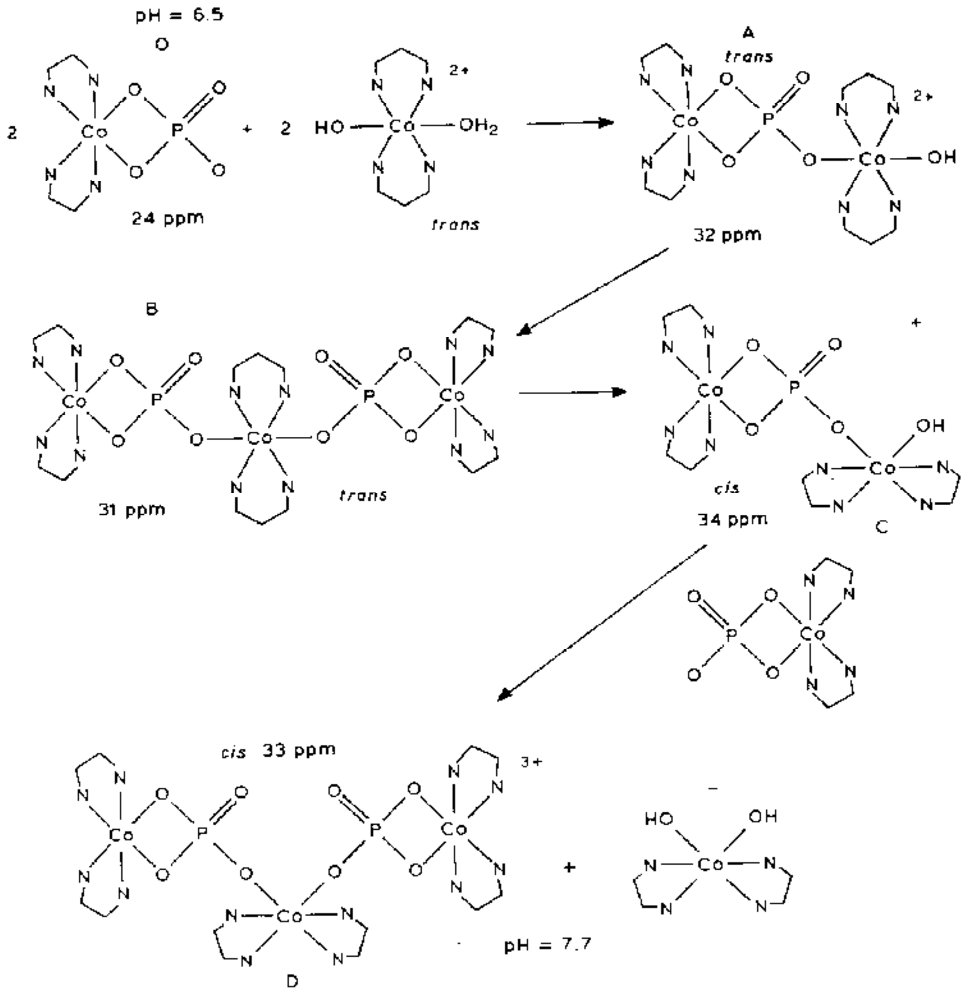
(II)

In 1979 Hubner and Milburn [11] reported a dramatic increase in the hydrolysis rate for pyrophosphate in the presence of $tn_2Co(OH)(OH_2)^{2+}$ at two or three times its concentration, with a 1 : 3 ratio deemed optimum. In 1984 our group reported [4] results, using NMR, of reacting $en_2CoP_2O_7^-$ (**II**) chelate with (**I**). A run from that study is presented in Fig. 6 together with Scheme 4, describing the course of reaction through intermediates, to yield a very specific product. Only one of seven possible ~ 33 ppm signals described in Fig. 5 is seen as a rather sharp singlet.

Several additional features to the reaction can now be described in addition to the optimum reactant ratio of 1 : 2, pH 7, first order kinetics for hydrolysis, and specific product previously discussed [14].

(a) Kinetics and stoichiometry

The kinetics and stoichiometry at pH 7 are very like those for V(V) and triphosphate, except that the second Co is necessary for hydrolysis to occur at all. Similarly, with a stoichiometric ratio of two (**II**) to one (**I**), no hydrolysis is observed: with a 1 : 1 ratio, about 25% hydrolysis occurs. 25% 1 : 2 Complex would be expected if (**I**) formed monodentate complexes completely with basic sites on (**II**). Such would account for the 25% hydrolysis in 1 : 1 solutions, the first order, 100% hydrolysis in 1 : 2 solutions, and the fact that lowering the ratio to 1 : 3 does not alter the rate of hydrolysis [14]. However, the presence of 4 ppm signals characteristic of free (**II**) or at least open basic sites on (**II**) during 1 : 2 runs seems to deny that possibility [4]. We suggest that the broadening of the 4 ppm signal is due to the formation of a very labile monodentate complex at all basic sites in bridged or chelated intermediates. If this is correct the behavior of (**I**) where



Scheme 3

it must form monodentate complexes is completely analogous to the behavior of VO^{3+} when it is bound to polyphosphates.

(b) Specific product

All attempts to isolate the specific product have failed [4,14], even though Fig. 5 strongly suggests that it is formed quantitatively. Every manipulation attempted caused it to dissociate to the $tn_2Co(PO_4)$ chelate. This fragility is further shown in Fig. 7 for a 1 : 2 run at 35°C [14].

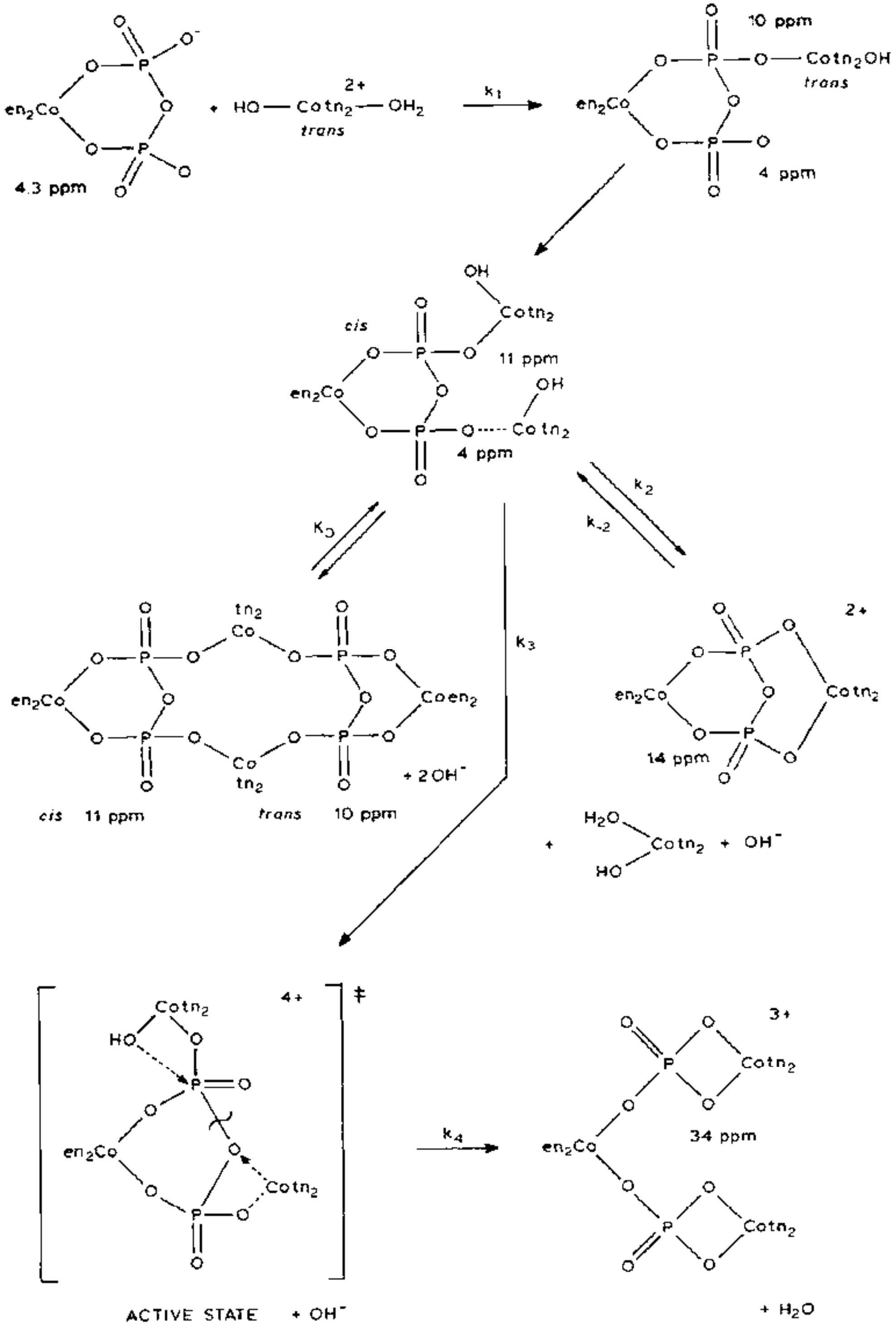
(c) Change in temperature (Fig. 7)

Increasing temperature above 25°C does not accelerate hydrolysis and may even slow it down if exothermic formation of complexes is sufficiently

Fig. 6. ^{31}P NMR signals vs. time for the reaction 0.05 M $en_2CoP_2O_7^-$ ($\partial = 4$ ppm) with 0.1 M $tn_2Co(OH(OH_2)^{2+}$ in 1.0 M TEA buffer at pH = 7, $T = 25\,°C$ (Scheme 3).

retarded. A ^{31}P NMR study of the hydrolysis of 0.05 M $tn_2CoP_2O_7^-$ by two moles of (**I**) at $T = 35\,°C$ shows the fragility of the single product observed at 33–34 ppm at 25° C. At the higher temperature hydrolytic opening of the six membered ring is accompanied by cleavage of a Co–O–P bond in the same ring and the formation of *trans*-$tn_2Co{=}O_2PO_2{-}Cotn_2{-}OH$ (or OH_2) corresponding to signal A in Fig. 5. The broad signal at $\partial \sim 7$–8 ppm in the 35 and 65 min spectra is typical in systems where Co(III) in (I) is being slowly reduced to paramagnetic Co(II). The broad signal at 28 ppm is consistent with three monodentate bonds of Co(III) to PO_4^{3-} or with three Co(III) bridges between two orthophosphates.

Lowering the temperature to 13° C slows down both complex formation and hydrolysis dramatically. We estimate values of activation parameters as $\Delta H^{\ddagger} = 38$ kcal and $\Delta S^{\ddagger} = +60$ e.u., characteristic of a highly organized activated state.


4.3 ppm
2+
HO — Cotn₂ — OH₂
trans
k₁
10 ppm
Cotn₂OH
trans
4 ppm
cis
Cotn₂
11 ppm
4 ppm
K₀
k₂
k₋₂
k₃
cis 11 ppm
trans 10 ppm
+ 2OH⁻
2+
14 ppm
+ H₂O Cotn₂ HO + OH⁻
4+
‡
ACTIVE STATE + OH⁻
k₄
3+
34 ppm
+ H₂O

Scheme 4

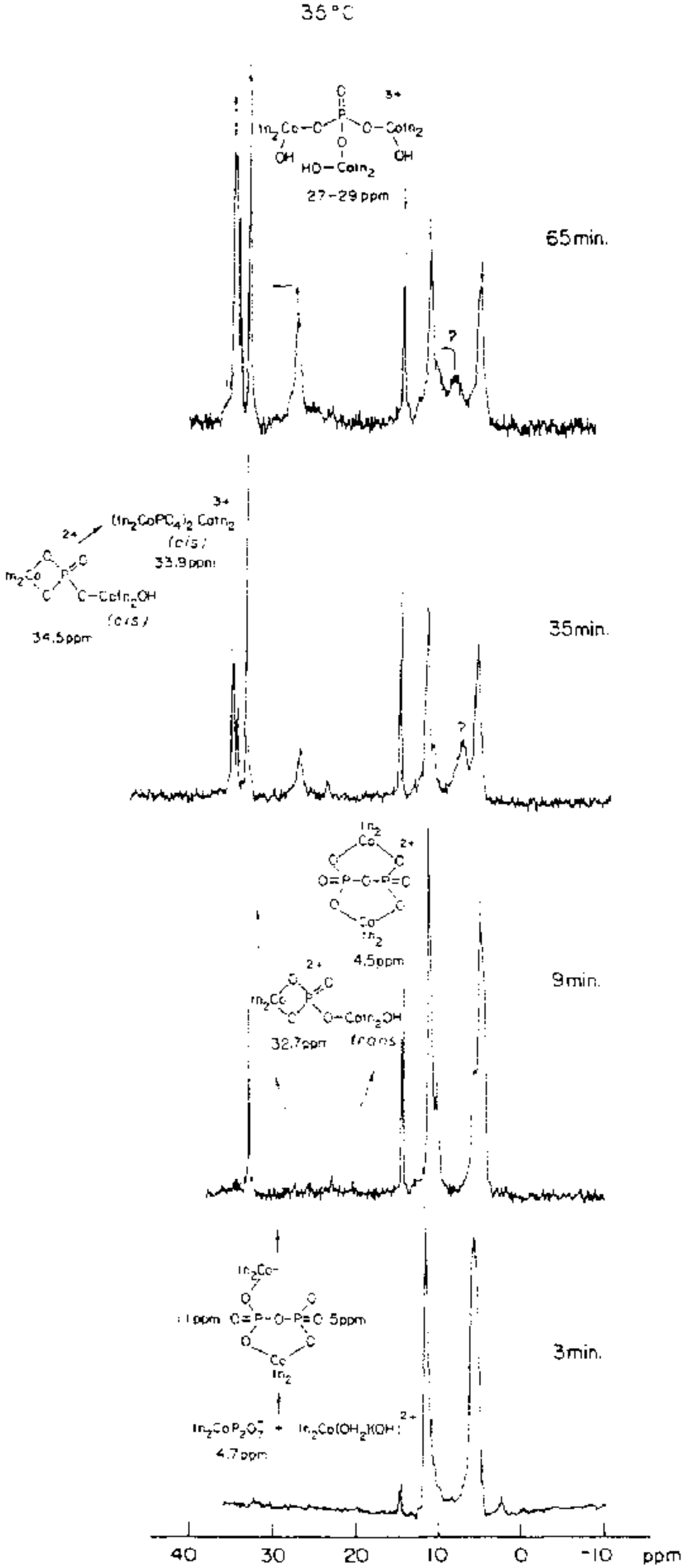


Fig. 7. Same reaction as Fig. 6 at $T = 25°$ C.

(d) Role of the second Co center in activation

The postulated role of the second Co(III) atom, to assist hydrolysis by electrophilic attack on the bridging O in P_2O_7 in (**II**), has been tested by reacting $(NH_3)_5CoP_2O_7^-$ chelated to (**I**) with a second molecule of (**I**) [4]. The monodentate complex formed has all the features of the activated state in Scheme 4 except that the pentammine Co(III) cannot be an electrophile or a nucleophile at pH 7. Hydrolysis in this system occurs, but roughly two orders of magnitude slower than for the reaction in Scheme [4].

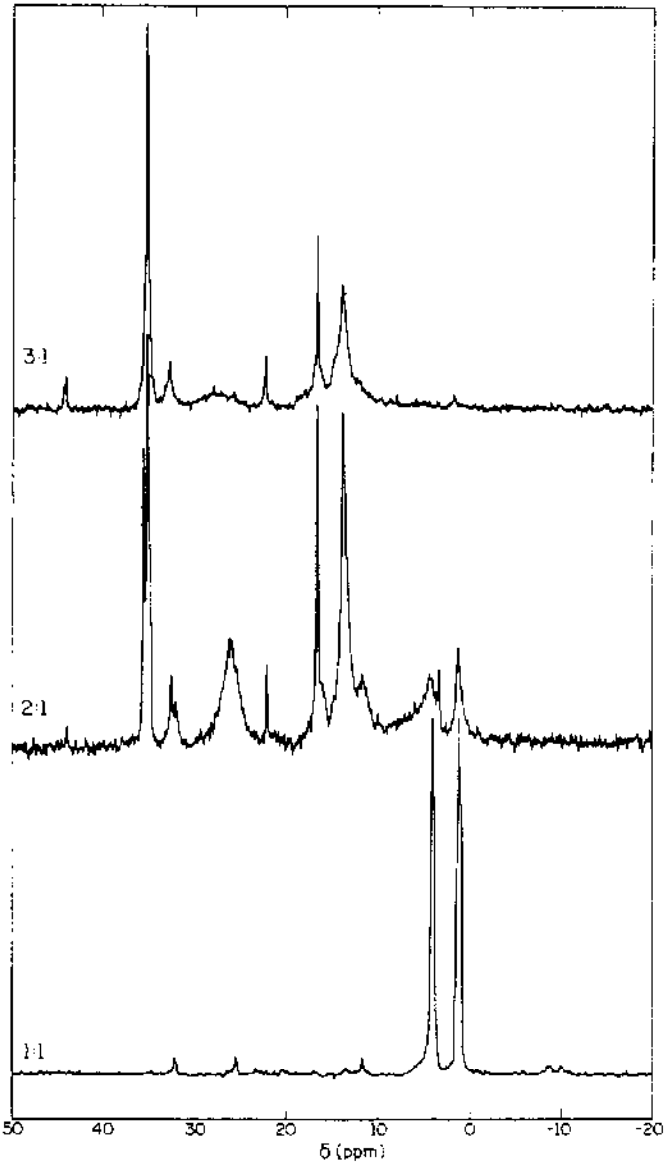
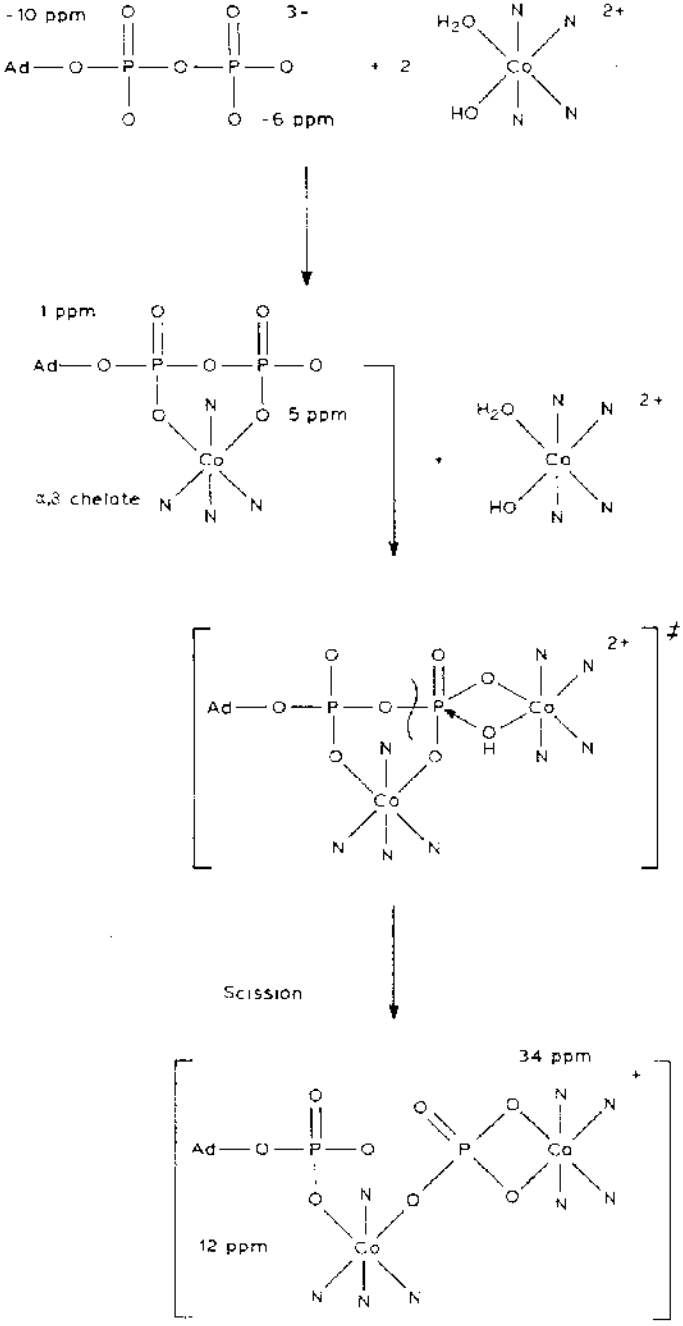


Fig. 8. ^{31}P NMR signals for the products of reaction of three successive additions of equimolar $tn_2Co(OH(OH_2)^{2+}$ to 0.1 M ADP (Scheme 4).

(iv) Reactions of (I) with a pyrophosphate monoester, ADP

Hediger and Milburn [17] observed in 1982 that ADP and ATP could each be hydrolysed by two moles of (I). We have found that at pH 7 careful addition of (I) to ADP produces quantitatively an α, β chelate in solution as



Scheme 5. Hydrolysis of ADP.

determined by NMR (Fig. 8) [15]. This complex is structurally analogous to the pentammine Co(III) complex described above, but in contrast, it is easily hydrolyzed by an additional molecule of (I). Adenosine appears to so activate the P–O–P bond as to remove the necessity of electrophilic attack on the bridging oxygen to assist (I) coordinated to the terminal PO_3^- in cleaving the $-O-PO_3^-$ bond. The acids of AMP, ADP, and ATP are relatively strong, making the basicity of –O–, to which PO_3^- is bound, relatively weak. Figure 8 and Scheme 5 show the end result of adding one, two, and three moles of (I) successively to ADP [15]. The second step clearly involves cleavage. A $tn_2Co(PO_4)$ chelate bound to the first tn_2Co is a principal product. This is not the exclusive product, however, indicating some reaction of (I) with cleavage products leaving some unreacted ADPCotn$_2$ at the end of the reaction. The third step consumes all remaining α, β chelate and produces a variety of products yet to be sorted out.

(v) Reactions of (I) with a pyrophosphate diester, NAD

In early studies with oxidized vanadium complexes, it was noted that hydrolysis of the diesters APA and APPA was much slower than that of ADP and ATP [3]. Further, NADH is often a reducing agent present in

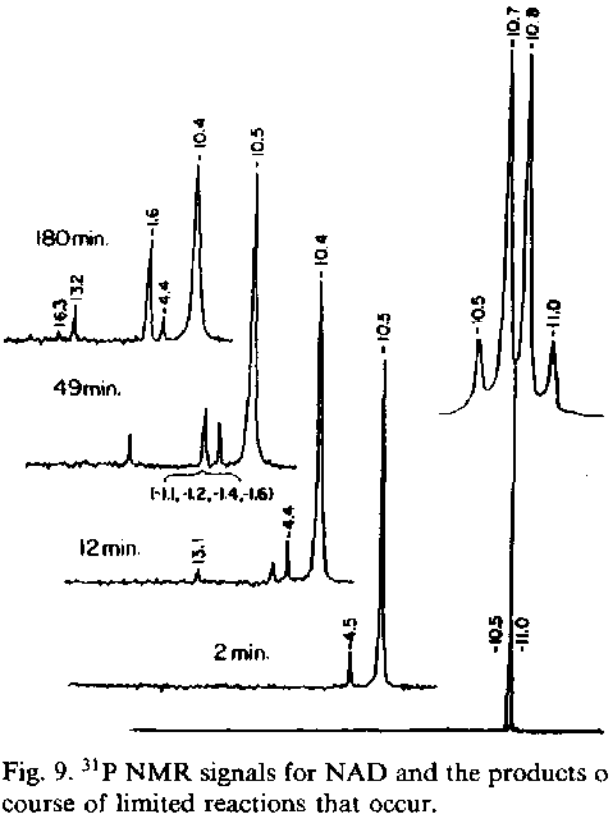
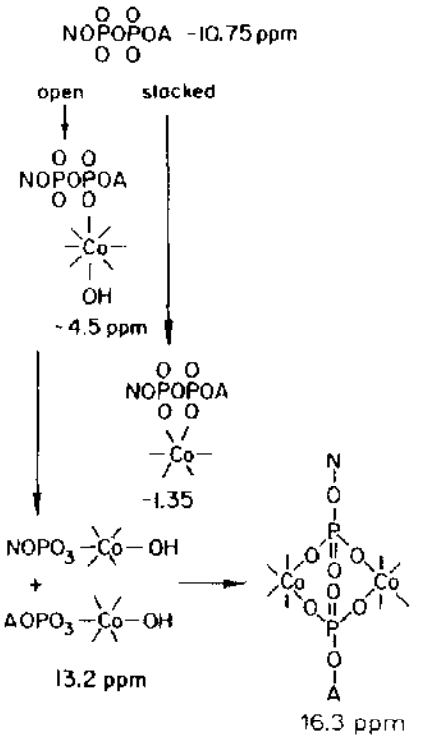


Fig. 9. ^{31}P NMR signals for NAD and the products of reaction with (I). Scheme 6 follows the course of limited reactions that occur.



Scheme 6

systems in which oxidative phosphorylation takes place. NAD is a dinucleoside with a pyrophosphate bridge similar to that joining the ends of complementary strands in the DNA double helix. It has but one basic oxygen on each end of the pyrophosphate. None of these pyrophosphate esters appear to undergo hydrolytic decomposition in biochemical systems where phosphorylation is taking place. The reactions of (I) with NAD are described in Fig. 9 and Scheme 6. The most striking feature of this system is the very limited amount of complex formation. Small signals do appear for monodentate Co–NAD (-4 ppm) and bidentate Co=NAD (-1.3 ppm), and corresponding monophosphate complexes form by hydrolysis of the monodentate species ($+13$ and $+16$ ppm). Also, significant broadening of the ^{31}P NMR signal for NAD suggests weak, labile coordination is occurring. Further investigation of this system will be made. It is quite likely that steric hindrance will prevent two molecules of (I) from coordinating as has been required for hydrolysis of all other pyrophosphates studied so far.

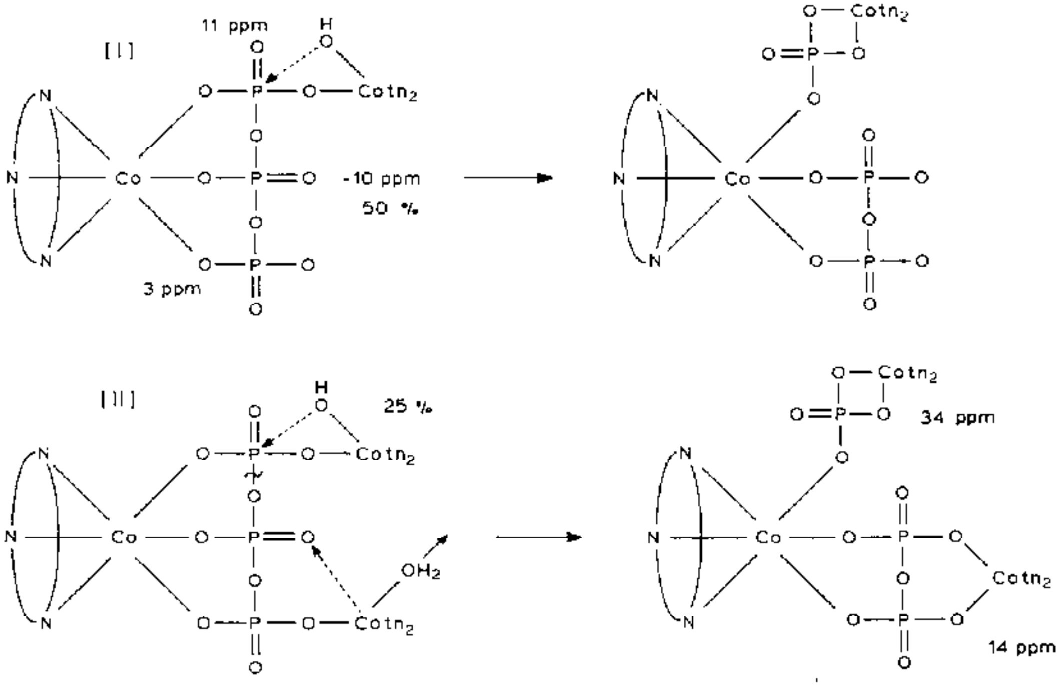
(vi) Protection of RO_3POPO_3R structures against hydrolytic decomposition in biochemical systems

NAD is too weak a base to be competitive with other phosphates in coordination to metal ions. This could very well be the source of its protection against hydrolysis in phosphorylating and hydrolyzing biochemical systems. This combined with steric hindrance, may well provide the necessary protection for pyrophosphate diesters in phosphorylating systems.

D. REACTIONS OF (I) WITH CHELATED TRIPHOSPHATES WITH AT LEAST ONE TERMINAL PO_3^- WITH ONE BASIC OXYGEN

(i) Reactions of (I) with a tridentate cobalt(III)-coordinated triphosphate, tacnCo(P_3O_{10})$^{2-}$

This complex [5] has two tridentate ligands coordinated to opposing faces on the octahedral Co(III) center (**I**), and like VO^{3+} on free triphosphate, can only coordinate monodentate to either, or both, of the terminal PO_3^- groups.

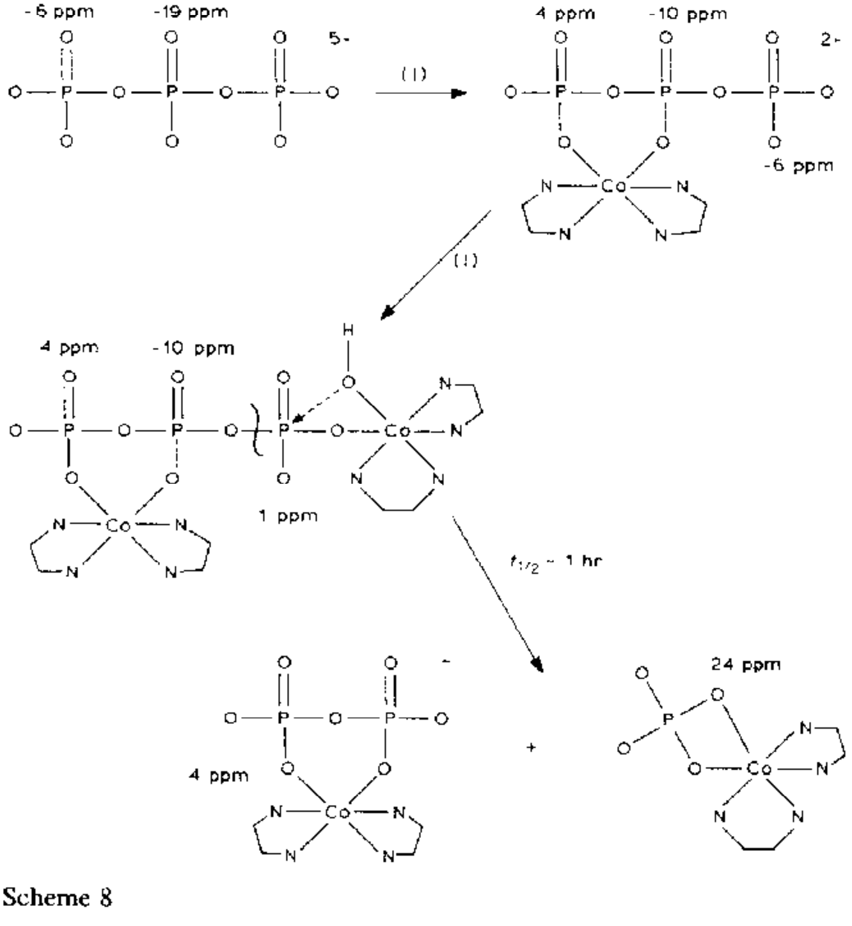


Scheme 7. Reactions in 1:1 solutions of (tacn)Co(PPP$_i$) (0.05 M) and $tn_2Co(OH)(OH_2)^{2+}$. Assume that $tn_2Co(OH)^{2+}$ binds monodentate to half the terminal PO_3^- sites in (tacn)Co(PPP$_i$)$^{2-}$. Statistically, this should produce 50% complex (**I**), 25% complex (**II**), and 25% free (tacn)Co(PPP$_i$). Complex (**I**) hydrolyzes as shown by a first order process, $t_{1/2} \simeq 3$ min. Complex (**II**) hydrolyzes as shown also with $t_{1/2} \simeq 3$ min. In this case the second $tn_2Co(OH)^{2+}$ coordinates to the newly basic oxygen atom on the β-phosphorus atom producing chelated PO_4^{3-} and $P_2O_7^{4-}$ both bound to (tacn)Co^{3+}. The free (tacn)Co(PPP$_i$)$^{2-}$ does not hydrolyze. Only 75% of the original (PPP$_i$) is hydrolyzed as observed [5]. In 1:2 solutions complex (**II**) forms completely and 100% hydrolysis of γ-PO_3^- is observed with $t_{1/2} \simeq 3$ min again.

This it does, producing complexes which undergo first order hydrolysis (P–O–P bond scission) with a half-time of about 3 min, the fastest such reaction yet observed [5]. In this case the two metal ions on either end do not double the rate of the hydrolysis, the second merely chelates to the newly basic middle P(V) center after hydrolytic cleavage at the other end. In contrast to results with chelated pyrophosphate, a galaxy of products is obtained with as many as 14 signals seen in the 31–35 ppm region of the NMR spectrum [5]. Scheme 7 shows the mechanism that has been deduced for this system.

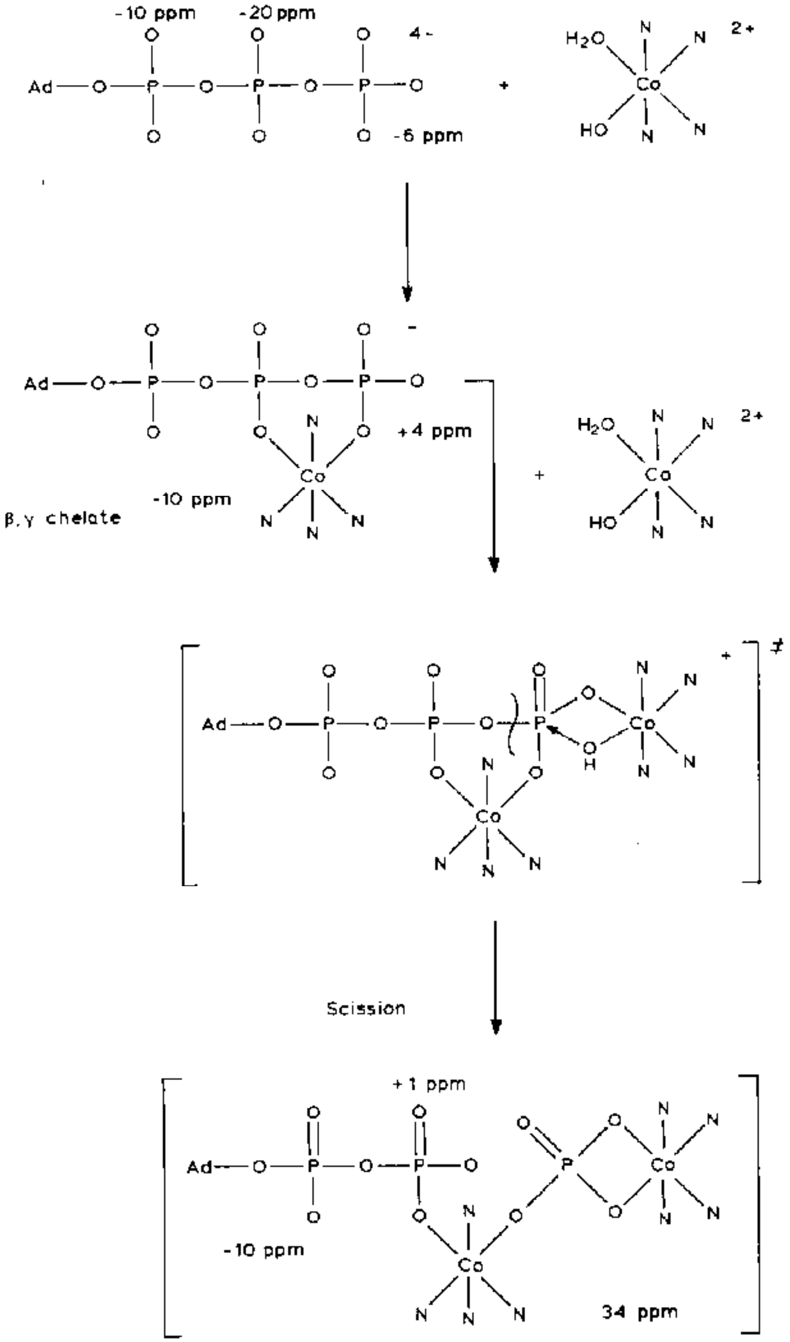
(ii) Reactions of (I) with other triphosphates

$P_3O_{10}^{5-}$, ATP, and cyclic $P_3O_9^{3-}$ are being examined by the technique applied to ADP (see Fig. 8. and Scheme 5). Preliminary results indicate that careful addition of equimolar (*I*) to these three species produces the α, β chelate of $P_3O_{10}^{5-}$, the β, γ chelate of ATP [17], and fast hydrolysis of two



Scheme 8

thirds of the cyclic $P_3O_9^{3-}$ to form a variety of complexes of P_i, PP_i, and PPP_i with one and two molecules of (**I**). 0.05 M Solutions of each reagent were used. Addition of a second mole of (**I**) to each system gave the following results.



Scheme 9

(a) Hydrolysis of α, β $tn_2CoP_3O_{10}^{5-}$ by (I) [18]

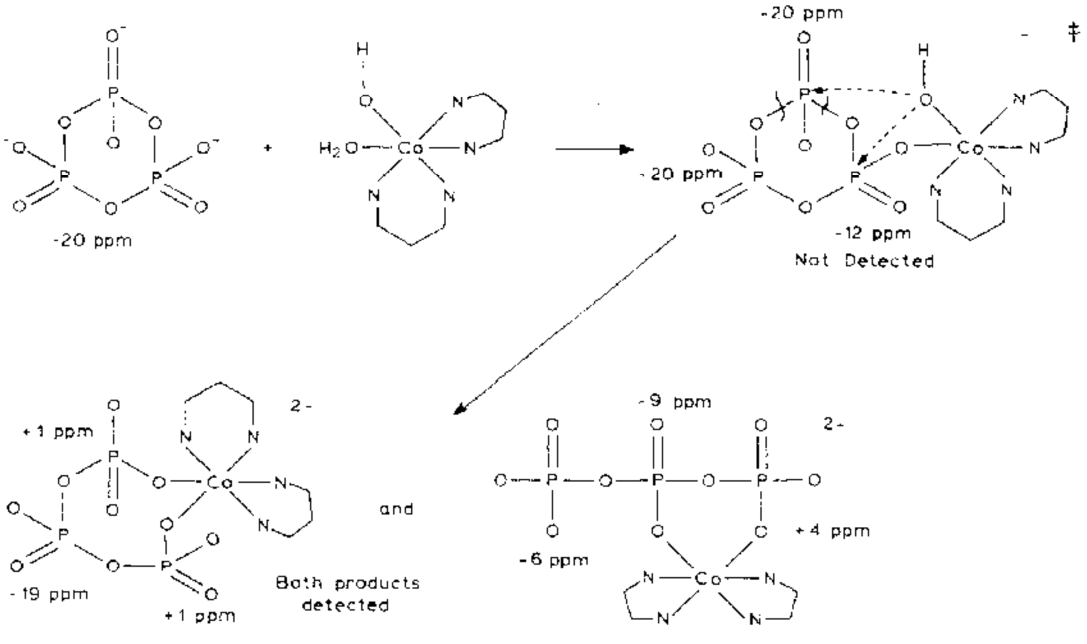
The major product of reaction of the chelate with a second molecule of (I) is tn_2CoPO_4 (24 ppm). This means that (I) reasonably coordinates monodentate to the most basic oxygen on the γ-phosphorus atom of the α, β chelate, and then hydrolyzes the terminal $O-PO_3^-$ bond, forming chelated tn_2CoPO_4. Hydrolysis is a first order process with a half-time of about 20 min. The mechanism is given in Scheme 8.

(b) Hydrolysis of the β, γ chelate of (I) with ATP

In this case, 34 ppm signals appear as soon as the second (I) is added cleaving the $\gamma-O-PO_3$ and forming a chelated PO_4^{3-} still bound monodentate to the first Co(III) center. Scheme 9 indicates the course of this reaction. It is interesting that the basic site on the α phosphorus is not attacked by (I). This site is both weakened as a base and sterically hindered by the adenosine ester bound to the α phosphorus.

(c) Hydrolysis of cyclic triphosphate, $P_3O_9^{3-}$

One cannot distinguish experimentally between: (1) scission caused by direct attack on a P(V) center by OH^- ligated to tn_2Co^{3+} followed by anation to give the two observed products; and (2) Scheme 10. In competition experiments with stronger bases ($P_3O_{10}^{5-}$, $P_2O_7^{4-}$, and HPO_4^{2-})$P_3O_9^{3-}$ is not hydrolyzed by (I). This suggests strongly that anation of (I) creates a



Scheme 10

complex which can hydrolyze P–O–P bonds as in all the other cases studied. In less than 2 min at least half the $P_3O_9^{3-}$ present is converted to the products shown. This sets an upper limit for the half-time for this hydrolysis in the range of seconds. The products are better bases than $P_3O_9^{3-}$, and prevent complete hydrolysis even after reaction with a second mole of (**I**).

E. DISCUSSION

Cornelius has given three mechanistic steps for acceleration of polyphosphate hydrolysis by inert metal ions (Co(III) and Pt(II)): (1) coordination of the metal ions to reduce the nucleophilicity of the polyphosphate; (2) coordination of a second metal ion having *cis* coordination positions available; (3) hydrolytic attack by the *cis*-hydroxide on the polyphosphate chain. We have shown variations on this theme, namely:

(a) Pyrophosphate requires two additional metal ions in step 2.

(b) Labile V(V) skips step 2 for triphosphates, because it is not able to form inert chelates which block step 3 in 1 : 1 complexes of N_4Co with polyphosphates.

(c) On the other hand labile V(V) requires the addition of 4 metal ions to pyrophosphate before step 3 can occur. Both labile V(V) and inert Co(III) must cover all basic oxygens in pyrophosphate. One Co(III) can tie up two sites by inert chelation, so one less metal ion is required. Recently Cornelius and co-workers [18] have reported hydrolyses of polyphosphates accelerated by inert, square planar, *cis*-$(NH_3)_2Pt(OH)(OH_2)^+$ (**III**). In this case some accelerated hydrolysis occurs even for 1 : 1 complexes in the presence of excess polyphosphate. This places (**III**) between Co(III) and V(V) although even V(V) acceleration is blocked by excess triphosphate. (**III**) seems unlikely to coordinate to more than one polyphosphate and that by chelation. However, substitution on inert square complexes is more facile than on inert octahedral complexes, such that a 1 : 1 complex of triphosphate with (**III**) could aquate at a moderate rate producing the *cis*-OH^- nucleophile to attack a P(V) center.

(d) Cyclic triphosphate ($P_3O_9^{3-}$) with (**I**) appears to go directly to step 3. No complex from step 1 is detected and ($P_3O_9^{3-}$) is not attacked competitively by (**I**) when more basic linear triphosphate and/or pyrophosphate are present.

Table 2 presents a list of metal polyphosphate complexes which undergo hydrolysis (postulated from kinetics and detected with ^{31}P NMR) together with estimated half times for their internal hydrolysis.

We do not pretend to be making model systems for enzymic phosphorylation reactions and hydrolyses. Clearly there are many factors available for activating and deactivating polyphosphates and for assisting and resisting

TABLE 2

Complexes which hydrolyze

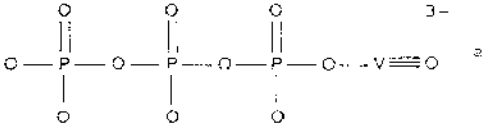
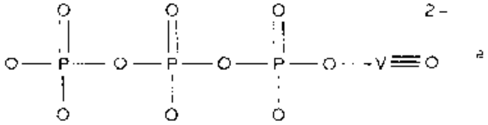
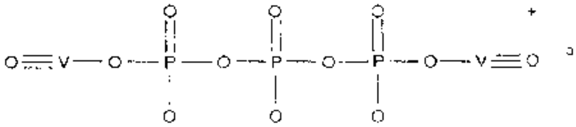
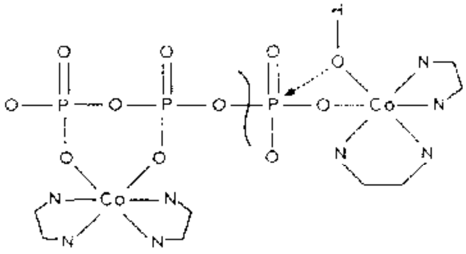
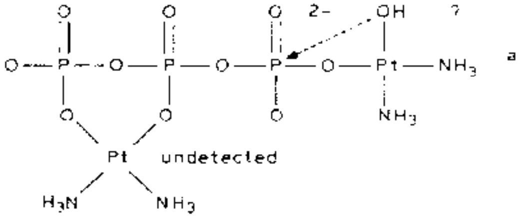
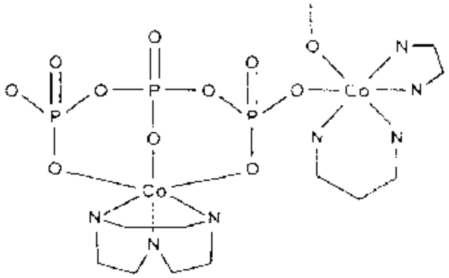
Complex	Approximate $t_{1/2}$	Ref.
Triphosphates		
	Days	3
	Minutes	3
	Minutes	3
	20 min	10, 19
	May be very fast (4 h) for formation and decay	18
	3 min	5

TABLE 2 (continued)

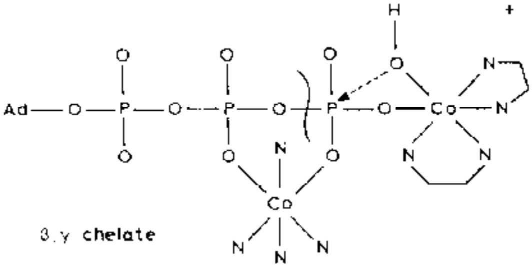
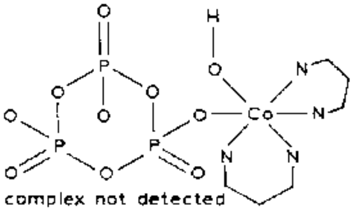
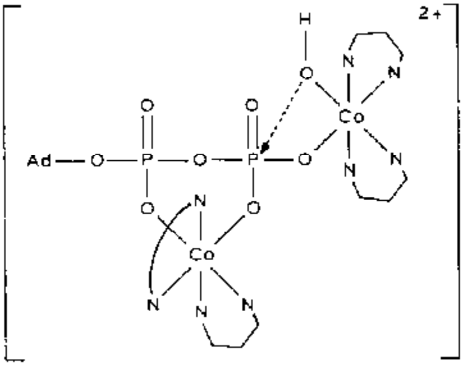
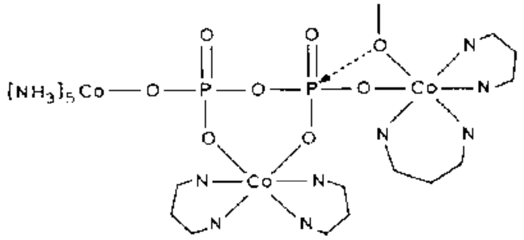
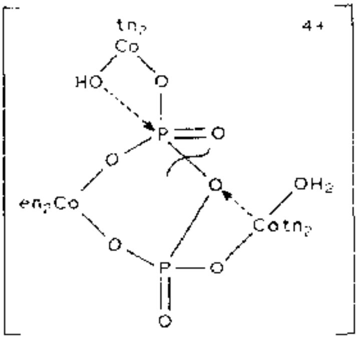
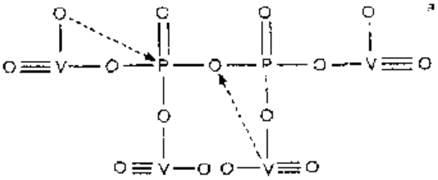
Complex	Approximate $t_{1/2}$	Ref.
 β,γ chelate	12 min	17
 [a] complex not detected	≥ Seconds	20
Pyrophosphates		
	20 min	15, 16
	Hours	4, 14

TABLE 2 (continued)

Complex	Approximate $t_{1/2}$	Ref.
	36 min	4
	~ 2 h	8

[a] Species postulated from kinetics, not detected with ^{31}P NMR.

complex formation by metal ions with the proper geometry and conditions for allowing such complexes to provide nucleophilic attack on P(V) centers. We have not as yet found a system which can use a phosphate as the nucleophile to transfer a PO_3 group to a phosphate or polyphosphate. Cornelius and Cleland [21] did report reversible phosphoryl transfer between $N_4Co(III)$–ATP complex and ∂-D-glucose-phosphate. We have been unsuccessful in attempts to attack $tn_2Co(PO_4H)(H_2O)^+$ in acid conditions to prevent formation of the chelate $tn_2Co(PO_4)$. Nucleophiles with which tn_2Co^{3+} can form bridges with polyphosphate complexes are being sought.

ACKNOWLEDGMENT

Thanks and credit are due all colleagues and students whose published and unpublished work I have cited.

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