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BIS(TRIMETHYLENEDIAMINE)COBALT(III) MEDIATED HYDROLYSIS OF TRIPOLYPHOSPHATE AND TRIMETAPHOSPHATE

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ABSTRACT. The hydrolysis of tripolyphosphate (PPPi)and trimetaphosphate (mPPPi) at 10^{-3} M concentration by $[(tn)_2Co(OH)(OH_2)]^{2+}$ (tn = trimethylenediamine), using Et /H quenching was studied. The reaction was monitored by measurement of all the phosphate species in the quenched aliquots of the reaction mixture of the reagents. A method was developed to separate and determine the different phosphate species after oxidizing them to orthophosphates and using acetonephosphomolybdate complex as the analytical reagent. The reactive species with regard to hydrolysis were found to be 3:1 $tn_2Co(III)$PPPi and $tn_2Co(III)$mPPPi complexes. The results showed larger amounts of hydrolytic products for the directly added systems than for those of stepwise additions. The production of phosphites in the reaction solution that contained trimetaphosphates was noted. A plausible mechanism for the reaction is forwarded. Quantitative determination of the phosphites was possible by oxidizing them with iodine to orthophosphates.

INTRODUCTION

It has been established that enzymes that catalyze the hydrolysis of phosphate esters, pyrophosphates and ATP require metal ions such as Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+} and Co^{2+} for their activity [1-3]. Divalent metal ions have been employed as catalysts for the hydrolysis of phosphate esters [4-7] with a view to revealing the mechanistic aspects and reasons for the rate enhancement during such reactions. The rapid enzymic hydrolysis of the P-O-P bonds in biological systems is some 10^{10} times faster than the slow hydrolysis of these bonds. For example under the in vitro conditions the hydrolysis of pyrophosphate by yeast inorganic pyrophosphatase (YIP), occurs about 10^{11} fold faster than the hydrolysis in the absence of the enzyme[8]. Several studies aimed at giving an insight in to the role of metal ions in the enzymatic reactions in biological processes have been published [9-15]. The major drawback with the class of divalent metal ions is that they are very labile towards solvolytic ligand displacement. Coupled to this, identification of species involved in the hydrolysis is marred by the presence of monomeric, dimeric and hydrated complexes [13].

In most model systems, the essential biologically active metal ions have been replaced by transition metal ions. The transition metals have the advantage of being fixed in their positions around the substrate molecule which in this case would be tetrahedral PO_4 structural units. The transition metals have been modified by introducing ligands such as amines on to the metal centre. This restricts the geometry and the number of coordination sites available for substrate models [14-17]. The choice of bis(trimethylenediamine)cobalt(III) was prompted by the fact that it retains its integrity for long periods of time and it is 100% cis in solution [18]. This would ensure maximum hydrolysis as it is envisaged that a complex with cis geometry has a better chance of hydrolysis as opposed to the corresponding trans isomer. Amine ligand effects in hydroxoaqua(tetramine) cobalt(III) promoted hydrolysis of ATP has been discussed in detail recently [19]. In the present work a modified analytical technique was developed to monitor all hydrolytic products formed in the reaction solution. This attempt is unique as earlier investigation were aimed at determining the amounts of orthophosphate ions produced in the solution without considering other phosphate species. The results of our investigation will provide a better understanding of the roles played by metal ions in phosphoryl transfer reactions.

EXPERIMENTAL

Analytical grade reagents were used except where otherwise indicated. Measurements of pH were made with an Orion ion analyzer model 901 using a combination electrode. The pH of the reaction mixture was maintained by adding drops of NaOH or $HClO_4$ from a glass rod. A Beckman DU 65 UV-Vis spectrophotometer was used to obtain spectra and collect rate data.

Synthesis of compounds. Hydroxoaquabis(trimethylenediamine)Cobalt(III) perchlorate was synthesized by employing the modified literature procedures [11,15,27,28].Tris(1,2-ethylenediamine)Cobalt(III) chloride was also prepared. Characterization of he compounds was possible by employing UV-Vis techniques. The molar extinction coefficients, and the absorption maxima and minima were found to correspond well to literature values [15].

Sodium tripolyphosphate and sodium trimetaphosphate were prepared and characterized using known methods [21,22,23]. The presence of orthophosphates as an impurity was detected in both tripoly-phosphate and trimetaphosphate by employing standard tests [20,25] to the extent of 2.35% and 1.94% respectively. The trimetaphosphate was found to be free of other condensed phosphate impurities, while in the tripolyphosphate the presence of pyrophosphate [26] was confirmed to the extent of 5.9%.

Determination of Pi. The amount of pi produced was determined by a modified Hirata and Appleman procedure [20]. The concentration of orthophosphate in the aliquots was found from calibration curve studies of standard solutions. In a typical protocol of the method, 3.2 mL of the sample was mixed with 0.8 mL molybdate reagent and after shaking thoroughly for one min, it was allowed to stand for an additional min. 4 mL of ice cold acetone was added to it using an automatic burette. The addition was done by inserting the tip of the burette below the surface of the solution. The resulting mixture was stoppered, shaken for one min to complete its color development and its absorbance measured at 328 nm. From an average of triplicate readings, calibration curve was drawn. The system obeyed Beer's law between the ranges 1 - 6 μg P/mL. The molar extinction coefficient was found to be $1.696 \times 10^4\ M^{-1}cm^{-1}$

Separation and determination of the various phosphates. A scheme for separation of the individual phosphate species in a solution of mixtures of Pi, PPI, PPPi, and mPPPi was developed as shown in figure 1. Standard solutions containing known amounts of the above mixtures were used as control and subjected to the analytical scheme. The experimental results agreed well with those of the expected values.

Hydrolytic studies. Solutions containing bis(trimethylenediamine)cobalt(III) and the condensed phosphates ($tn_2CoPPPi$ and $tn_2ComPPPi$) were prepared with metal to phosphate ratio of 1:1, 1:2, and 1:3 in such a way that the final concentration would be 1×10^{-3} M in the desired complex.

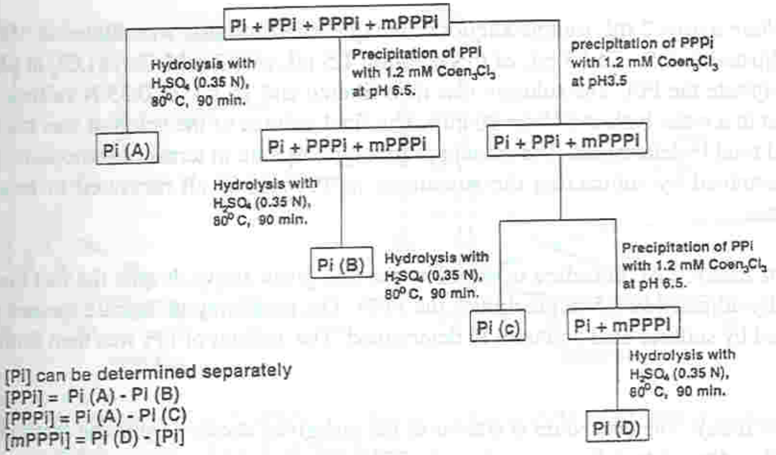


Figure 1. Assay for condensed phosphates.

1 M $NaClO_4$ was used for ionic strength control so that the solution would be 0.1 M in $NaClO_4$. During the course of the reaction the solution was continuously stirred using a magnetic stirrer. An example of the protocol for the preparation of a solution of 1 x 10^{-3} in $tn_2CoPPPi$ (1:1 metal to PPPi) is given here. 8 mL of PPPi (5 x 10^{-3} M, pH 6.5) was mixed with 4 mL of $NaClO_4$ (1 M, pH 6.5 and 20 mL of distilled water. The pH of this solution was adjusted to 6.5 using glass stick dotting. 8 mL of the cobalt complex ($tn_2Co(OH)(OH_2)$, 5 x 10^{-3} M, pH 6.5) was added dropwise to the above solution with continuos stirring. The final volume was 40 mL. For the higher metal to phosphate ratios, the amount of water was reduced accordingly to maintain the final volume to 40 mL. The hydrolysis was then monitored by correlating the amount to Pi, PPi, PPPi, and mPPPi in the solution with time. 2 mL aliquots were withdrawn at different time intervals (2, 5, 10, 20, 30, and 60 min) and quenched with Eu(II) to stop the reaction and release all bound phosphate species before assaying [14, 15]. For the determination of total phosphates, 2 mL aliquot of the quenched solution was diluted to 100 mL and its pH adjusted to 6.5. 75 mL of this solution was then take and 15 mL of 0.35 N H_2SO_4 added. The solution was kept in a water bath at 80 °C for a period of 90 min to effect hydrolysis of all condensed phosphates to orthophosphates. Finally the volume was made up to 100 mL (pH 6.5) and spectrophotometric determination of pi conducted. The experimental results agreed well with those of the expected value for the total phosphates in the aliquots.

Trimetaphosphate assay. 2 mL aliquot of the quenched solution was diluted to 100 mL and its pH adjusted to 3.5 with perchloric acid. To 75 mL of the aliquot, 2.5 mL of 1.2 mM $Co(en)_3Cl_3$ (pH 3.5) was added to precipitate PPPi. After filtering the pH was adjusted to 6.5 with sodium hydroxide. Excess $Co(en)_3Cl_3$ at pH 6.5 was added to precipitate any PPi present. The solution was then filtered and 15 mL 0.35 N sulfuric acid added and heated in a water bath for 90 min at a temperature of 80 ° to hydrolyze the mPPPi. The final volume was adjusted to 100 mL and the amount of mPPPi measured in terms of Pi. The concentration of mPPPi was determined by subtracting the free pi preset in the solution from the final measurement. The amount of free Pi was initially determined from the unhydrolyzed filtrate.

Tripolyphosphate assay. 2 mL sample aliquot of the quenched solution was diluted to 100 mL and its pH adjusted to 6.5. To 75 mL of this solution 1.5 mL of 1.2 mM $Co(en)_3Cl_3$ at pH 6.5 added to precipitate the PPi. The solution was then filtered and 15 mL of 0.35 N sulfuric acid added and kept in a water bath at 80 ° for 90 min. The final volume of the solution was made up to 100 mL and total Pi determined. The amount of tripolyphosphate in terms of orthophosphates was then determined by subtracting the amount of mPPPi and Pi all measured in terms of orthophosphates.

Pyrophosphate assay. The procedure is similar to the one given above despite the fact that the pH was initially adjusted to 3.5 to precipitate the PPPi. The remaining phosphate species were then hydrolysed by sulfuric acid and total Pi determined. The amount of PPi was then found by difference.

Pyrophosphate assay. The procedure is similar to the one given above despite the fact that the pH was initially adjusted to 3.5 to precipitate the PPPi. The remaining phosphate species were then hydrolysed by sulfuric acid and total Pi determined. The amount of PPi was then found by difference.

RESULTS AND DISCUSSION

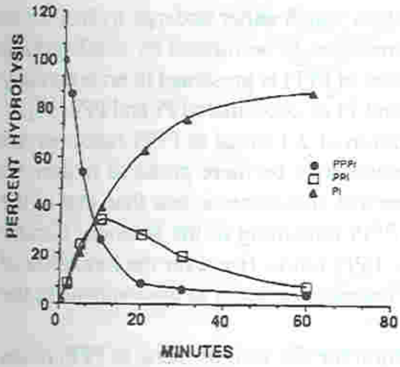
The results for the hydrolytic reactions of PPPi and mPPPi by $tn_2Co(III)$, pH 6.5, Eu(II) quenching are given in figures 2 and 3. Values in the figures are expressed as percentages measured in terms of orthophosphates.

As depicted in Figure 3, the total sum of the hydrolytic products of mPPPi do not add up to 100%. It is also evident that there was steady decrease in the total amount of phosphate during the course of the reaction. Qualitative tests [22] were carried out to see if other oxy-acid salts of phosphorous were formed. The result showed the presence of phosphites in the solution. Quantitative analysis was then carried out by oxidizing the orthophosphites to the corresponding orthophosphates [30] with iodine and measuring the resulting orthophosphate. The result of the investigation is depicted in Figure 4.

Note that with the addition of the phosphite, the total sum for the hydrolysis products for mPPPi adds up to 100%.

From the results presented, salient features have been noted. The reactive species, $[tn_2Co(OH)OH_2)]^{2+}$, predominates at pH 6.5 [11,15]. The molecule has a coordinated nucleophile (OH) and a labile H_2O attached to the cobalt centre. It has been observed that a coordinated hydroxide is a very effective nucleophile in the intramolecular hydrolysis of nitriles, olefins, amino acid esters [23] and phosphate esters [9,11,15]. The reactivity of the hydroxoaqua complex has been attributed to the electrons freed from the diaqua complex by loss of a proton. This rings of the trimethylenediamine moieties around the cobalt centre has been presumed to enhance the reactivity of the coordinated hydroxide.

In the hydrolysis of both PPPi and mPPPi, it was noted that the rate of hydrolysis increases as the metal to condensed phosphate ratio increases. The highest rates were observed for the direct addition 3:1 ratio. This is evidenced by the highest production of hydrolytic products and low concentrations of residual PPPi and mPPPi remaining in the solution during the course of the



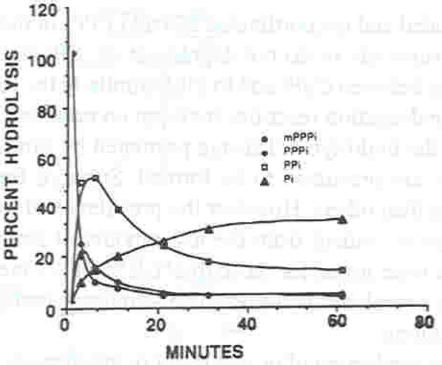


Figure 2. Hydrolysis of tn$_2$CoPPPi (10^{-3} M) at 3:1 Co to PPPi ratio (direct mixing, pH = 6.5, at 25°, I = 0.1 M NaClO$_4$).

Figure 3. Hydrolysis of tn$_2$ComPPPi (10^{-3} M) at 3:1 Co to mPPPi ratio (direct mixing, pH = 6.5, at 25°, I = 0.1 M NaClO$_4$).

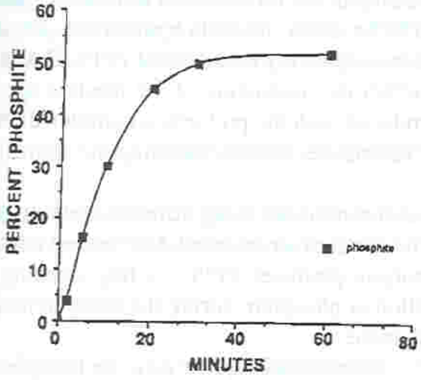


Figure 4. Production of phosphite as function of time in tn$_2$ComPPPi (10^{-3} M) reaction solutions (at 3:1 Co to PPPi ratio, direct mixing, pH = 6.5, at 25°, I = 0.1 M NaClO$_4$).

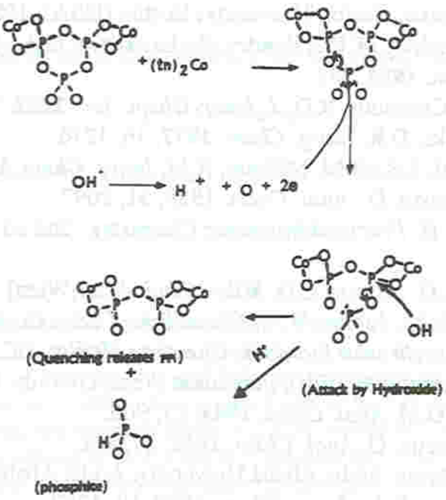
reaction. This observation was found to be in line with the notion that two or metal ions per mole of substrate are essential for effective hydrolysis of condensed phosphates [28, 32-34]. The 3:1 metal to condensed phosphate ratio causes high charge neutralisation in the coordinated phosphates. Hence this could contribute to the high rate of hydrolysis observed. The hydrolytic cleavage promoted by the 1:1 metal to PPPi ratio initially involves the formation of the monodentate CoPPPi, which can then undergo further reaction giving four, six and eight membered ring systems analogous to what was proposed in related studies [35]. Some of these reactions would lead to the formation of the chelate rings accompanied by hydrolytic cleavage while others form less reactive (stable) chelate rings which do not undergo further cleavage. Earlier ^{31}P NMR studies have shown that the six membered ring systems were more prevalent than either the four or eight membered chelates [28]. Appreciable amounts of PPi and PPPi remain in the solution during the course of the reaction. This can be attributed to the presence of coordinated and uncoordinated PPi and PPPi in the solution which either undergo hydrolysis at a very slow rate or do not degrade at all. PPi is also presumed to be formed by condensation reactions between CoPi and Pi [36]. Similarly the formation of PPPi is presumed to arise partially from condensation reactions between coordinated PPi and Pi or coordinated Pi and PPi [37].

In the hydrolytic cleavage promoted by direct addition of 2:1 metal to PPPi ratio, several chelates are presumed to be formed. Some of the chelates may be more prone to hydrolytic cleavage than others. However the prevalence of less reactive complexes is less than that of the 1:1. This is evident from the low amount of residual PPPi remaining in the solution. Similar features were noted for those of 1:1, 1:2 ad 3:1 metal to PPPi ratios. However the formation of reactive complexes that presumably undergo complete degradation seem to predominate in the 3:1 solutions.

The production of pi was lower in the stepwise addition for the various metal to PPPi ratios compared to those of direct mixing. This could be attributed to the formation of predominantly reactive chelates for the direct mixed systems which eventually undergo complete degradation. Addition of the cobalt complex to PPPi at 1:1 molar ratio results in rapid water substitution to form the monodentate complex which eventually forms ring systems. The rate of formation is presumed to be relatively fast. Addition of another molar ratio of the cobalt solution to the preformed 1:1 complex at pH 6.5 results in the formation of several chelates out of which the predominant species are presumed to be stable towards hydrolysis. For the direct addition systems more than one metal ion reacts with every molecule of PPPi. The random attack of the metal ion on the PPPi would then effect the production of the reactive species. In the case of stepwise additions, the first bond produced with the preformed complex dictates the position to which the second metal ion would coordinate, thereby effecting the formation of less reactive chelates.

The hydrolysis of mPPPi was also carried out using different metal to mPPPi ratios. From the study it is apparent that two distinct features are revealed. One feature conforms to the general observation [35] that mPPPi hydrolysis produces PPPi via ring opening which eventually degrades to PPi and Pi. The production of phosphite during the reaction prompted us to devise a mechanism which is depicted in Scheme 1.

Upon coordination of the metal to the trimetaphosphate ring, the phosphorous atom or atoms to which the metal is directly bonded via an oxygen bridge is made relatively positive. this will create an inductive effect making the uncoordinated phosphorus also relatively positive (i.e electrophilic). The electrons that are freed by the oxidation of hydroxide would then attack this electrophilic phosphorous atom thereby promoting ring cleavage and forming a nucleophilic phosphorous center. The nucleophilic phosphorous will eventually accept protons from the solution leading to the production of phosphites. Evidence for the above mechanism stems from the fact that bubbles of oxygen gas were detected during the hydrolysis of mPPPi. Furthermore the amount of PPi produced during hydrolysis of mPPPi is more than that obtained from PPPi The additional PPi can be rationalized as production of one mole of phosphite also releases an equivalent amount of PPi as shown by the mechanism. An increased degree of hydrolysis was observed for 3:1 metal to mPPPi ratio, and within the 3:1 ratios, the highest hydrolytic cleavage was noted for the direct added systems. This observation is in line with that noted for PPPi. The role of the metal ions in both cases is attributed among other factors to activation, substrate formation and structural consideration.



Scheme 1. Suggested features for the mechanism of phosphite generation in $tn_2Co(III)$-promoted hydrolysis of mPPPi. 3;1 Co to mPPPi ratio, pH 6.5, ambient temperature and 0.1 M $NaClO_4$ ionic strength. Charge and degree of protonation omitted.

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