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Cu(II) Catalysed Hydrolysis of Thiaminepyrophosphate: Structure-Reactivity Relationship

BADAR TAQUI KHAN* and P. NAGESWARA RAO

Department of Chemistry, Osmania University, Hyderabad-500007, India

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Abstract

The kinetics of the Cu(II) catalysed hydrolysis of thiamine-pyrophosphate (TPP) has been studied in aqueous solution at 56, 64 and 78° over a pH range of 3.0 to 7.0 at a constant ionic strength of 0.10 M (KNO_3). The pH rate profiles were analysed and the overall rate constants resolved into individual specific rate constants relating to various Cu:TPP chelate species in solution. Activation parameters $\Delta H^\ddagger$, $\Delta S^\ddagger$ and $\Delta G^\ddagger$ for the specific rate constants of various chelate species of TPP are reported. The possible mechanism of the Cu(II) promoted hydrolysis of TPP is discussed. The structure-reactivity relationship is also discussed.

Introduction

Thiaminepyrophosphate (TPP/Cocarboxylase) is the coenzyme for decarboxylases. Phosphate transfer reactions are of vital importance in biological systems and are catalysed by enzymes which generally require bivalent metal ions. The study of the mechanism of metal ion catalysis in simple nonenzymic systems will be of much help in understanding the more complicated reactions occurring during the metabolism of phosphate esters and the role of metal ions in the enzymic phosphate reactions. There are a few reports in the literature on the hydrolysis of some nucleoside phosphates [1–3]. In our earlier publication a detailed kinetic study on the spontaneous hydrolysis of TPP was reported [4]. In the present paper a detailed mechanistic study of the metal ion catalysed hydrolysis of TPP at 56, 64 and 78° over a pH range of 3.0–7.0 at constant ionic strength of 0.1 M (KNO_3) is reported.

Experimental

TPP (as hydrochloride) used in this investigation was obtained from United States Biochemical Corporation, U.S.A. The aqueous solution of copper nitrate (Analar grade) was standardised using the disodium salt of EDTA by the procedure outlined by Schwarzenbach [5].

The experimental procedure used in this investigation was essentially the same as that reported for the study of the uncatalysed hydrolysis of TPP [4]. The experiments were carried out at 56, 64 and 78°, and at constant ionic strength using 0.1 M KNO_3. The pH of the solution was maintained constant at the required value by the addition of acid or alkali during a kinetic run. No buffers were used, to avoid the interaction of Cu(II) with other ligands.

Kinetic runs were carried out with equimolar amounts of Cu(II) and TPP (2×10^{-3} M), over the pH range 3.0–7.0, at 56, 64 and 78°. First order rate constants were evaluated in the initial stages of the hydrolysis reactions from plots of $\log a/a - x$ against 't' (where a = initial concentration of TPP and x = amount reacted at time t). The analysis of samples for inorganic orthophosphate involves the precipitation of orthophosphate with the help of calcium chloride. The precipitate of calcium phosphate was then separated by centrifugation, dissolved in 60% perchloric acid and was used for the determination of orthophosphate by the spectrophotometric technique as reported earlier [4].

Results and Discussion

The effect of Cu(II) ions on the hydrolysis of TPP in 1:1 molar ratio of metal ion to TPP was studied. First order rate constants for the hydrolysis of TPP in the presence of Cu(II) were evaluated and listed in Table I. Kinetic runs were carried out over a pH range of 3.0–7.0 to study the effect of change in pH on the Cu(II) catalysed TPP hydrolysis. The rate of hydrolysis of TPP was found to increase with increasing pH up to pH 5.5 and then decrease with increasing pH (Table I). The rate of hydrolysis in the presence of Cu(II) was found to be higher than the rate of spontaneous hydrolysis of TPP over the entire experimental pH range. The concentration of the different chelate species of Cu(II): TPP in solution were

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*Author to whom correspondence should be addressed.

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TABLE I. First Order Rate Constants for the Cu(II) Catalysed Hydrolysis of TPP.

pH	56 °C		64 °C		78 °C	
	k_{obs} $s^{-1} \times 10^6$	k_{calc} $s^{-1} \times 10^6$	k_{obs} $s^{-1} \times 10^6$	k_{calc} $s^{-1} \times 10^6$	k_{obs} $s^{-1} \times 10^6$	k_{calc} $s^{-1} \times 10^6$
3.00	0.33	0.33	0.64	0.64	1.72	1.72
4.00	1.05	1.16	2.23	2.27	4.93	5.02
5.00	1.73	1.85	3.48	3.57	9.66	9.81
5.50	8.75	2.19	19.30	4.26	48.03	11.66
6.00	5.82	1.14	12.82	2.73	31.95	7.50
7.00	2.67	0.08	5.87	0.15	14.64	0.33

TABLE II. Mole Fractions of the Cu(II): TPP Chelate Species in Solution at 56°.

pH	MHL	ML
3.00	0.170	–
4.00	0.460	0.10
5.00	0.295	0.490
5.50	0.135	0.745
6.00	–	0.545
7.00	–	0.030

TABLE III. Specific Rate Constants (sec^{-1}) for the Cu(II) Catalysed Hydrolysis of TPP.

Species	56°	64°	78°
$CuTPPH^+$	1.97×10^{-6}	3.89×10^{-6}	10.87×10^{-6}
CuTPP	2.58×10^{-6}	5.09×10^{-6}	14.2×10^{-6}

TABLE IV. Activation Parameters for the Cu(II) Catalysed Hydrolysis of TPP.

Species	$\Delta H^{\ddagger}$ (kcal/mole)	$\Delta S^{\ddagger}$ (56°) (eu)	$\Delta G^{\ddagger}$ (56°) (kcal/mole)
$CuTPPH^+$	17.41	−12.4	21.50
CuTPP	17.27	−11.2	20.95

calculated using the thermodynamic data reported earlier [6]. The corresponding mole fractions at 56° are presented in Table II.

The overall experimentally obtained first order rate constants were resolved into specific rate constants relating to the catalytically active monoprotonated ($CuTPPH^+$) and normal (CuTPP) chelate species at different temperatures and are listed in Table III. From the evaluation of specific rate constants for the Cu(II), chelate species and the mole fractions of different chelate species a theoretical pH-rate profile was drawn. The observed and the calculated rate constants are in good agreement up to pH 5.0 (Table I). The deviation at higher pH values may be due to hydrolysis of Cu(II) chelate species. Activation parameters $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta G^{\ddagger}$ related to the specific rate constants of the catalytically active Cu(II): TPP chelate species ($CuTPPH^+$ and CuTPP) were evaluated by studying the kinetics of the Cu(II) catalyzed TPP hydrolysis at 56, 64 and 78°. The activation parameters are given in Table IV. Cu(II) interacts with TPP to form the protonated ($CuTPPH^+$) and the normal (CuTPP) chelate species as shown by potentiometric study. The copper complex of TPP exists predominantly in the form $CuTPPH^+$ and CuTPP up to pH 5.50. In the pH region 3.0–5.50 the observed rate can be assigned to these predominant Cu(II) chelate species. The rate law for the hydrolysis of TPP in the presence of Cu(II) is given by the relationship:

$$\frac{-dL}{dt} = k_{obs}T_L$$

where T_L = $CuTPPH^+$ + CuTPP, and $k_{obs} = k_1M_1 + k_2M_2$.

k_{obs} is the overall catalytic rate constant after subtracting the rate for spontaneous hydrolysis, k_1 and k_2 are the specific rate constants and M_1 and M_2 are the mole fractions of the $CuTPPH^+$ and CuTPP species, respectively. It is observed that Cu(II) catalyses the rate of TPP hydrolysis considerably as shown by the comparison of the specific rate constants of $CuTPPH^+$ and CuTPP with those of the corresponding free ligand species, $TPPH^-$ and TPP^{2-} [4]. Metal ion catalysis is most effective in the case of the normal chelate species (CuTPP).

The catalytic activity of the Cu(II) ions on the rate of TPP hydrolysis can be directly attributed to the changes brought about in the charge of the TPP molecule and polarization of the P–O bonds. On coordination with the TPP molecule, Cu(II) reduces the negative charge on the molecule by two units. Due to this decrease of the negative charge on the oxygen atoms of the phosphate chain, the approach of a nucleophile to the site of attack is facilitated

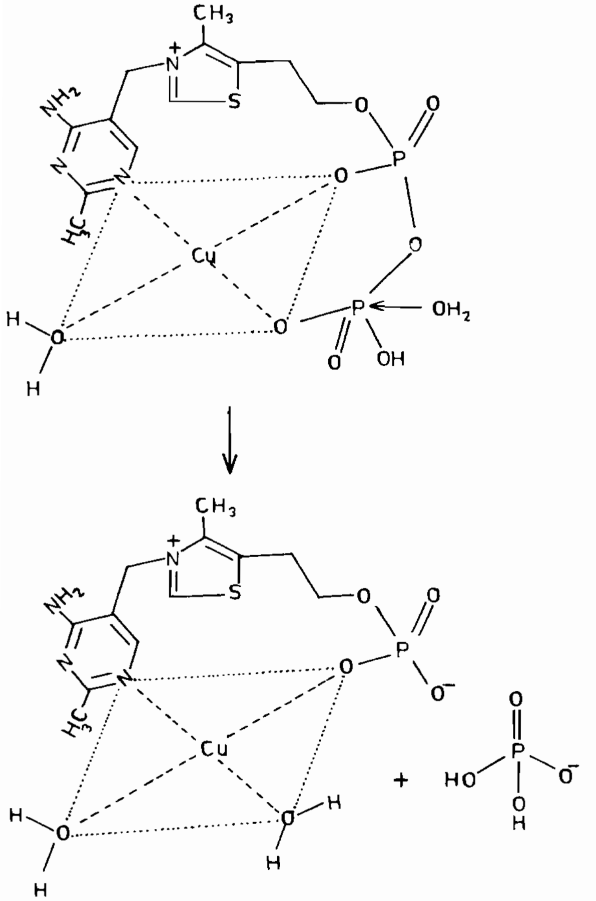

Fig. 1. Mechanism of Cu(II) catalysed hydrolysis of TPP (CuTPPH$^+$).

leading to an enhanced rate of hydrolysis. Further, the coordination of the Cu(II) ion with the oxygen atoms of the phosphate chain results in the withdrawal of electrons from the oxygen atoms towards the sites of metal binding. This leads to the polarization of the P–O bond, making the phosphorus atom susceptible to nucleophilic attack by a polar water molecule.

It was observed that the normal complex species CuTPP reacts at a faster rate than the monoprotonated species CuTPPH$^+$ as indicated by the data in Table I. In the monoprotonated species there is a possibility of the β-phosphorus atom (the site of attack) being polarized by both the metal ion and the proton. However, the greater interaction of Cu(II) in CuTPP species (log K_{ML} = 5.21 at 56°) is more than enough to compensate for the combined polarizing effect of CuTPPH$^+$ species (log K_{MHL} = 3.70 at 56°). The greater the interaction of the metal ion with TPP the higher is the polarization of the P–O bond and the consequent activation of the phosphorus atom towards the attacking nucleophile.

The enthalpies in the presence of Cu(II) are less endothermic than those for the spontaneous hydrolysis of TPP [4]. This is clear evidence for the catalysis of the hydrolysis of Cu(II) ions, since hydrolysis in the presence of Cu(II) requires less energy. However, the protonated (CuTPPH$^+$) and normal (CuTPP) chelate species undergo hydrolysis involving the same amount of energy as indicated by the similar enthalpy of activation of these two species (Table IV). The same trend was observed in the case of ATP [2].

The hydrolysis of the monoprotonated and the normal copper chelates of TPP is associated with large negative entropies of activation (Table IV). The large negative entropies are associated with hydrolysis reactions that proceed by an SN_2 bimolecular mechanism [7]. The monoprotonated uncomplexed TPPH$^-$ species may undergo hydrolysis by an SN_1 route involving internal proton transfer through a cyclic six membered ring formation and elimination of the metaphosphate anion. In the corresponding monoprotonated metal complex MTPPH$^+$ the negative charge on the β-phosphate, which is the driving force behind the SN_1 mechanism for the TPPH$^+$ species, is absent. Further, the complexation of the metal ion with the oxygens of the α and β-phosphates hinders the free rotation of the terminal phosphate moiety and the transfer of proton to the leaving group required for the SN_1 mechanism is thus rendered difficult. For these reasons the monoprotonated CuTPPH$^+$ species seems to favour an SN_2 route for its hydrolysis (Fig. 1).

The hydrolysis of the normal chelate species CuTPP by an SN_1 mechanism with the elimination of metaphosphate can also be ruled out, since the coordination of the metal ion to the α and β-phosphate oxygens facilitates the nucleophilic attack, but would hinder the generation of metaphosphate anion, which seems to be stabilized by coordination to the metal ion [8, 9]. A metaphosphate elimination mechaninsm would be possible if the metal ion is bound to the α-phosphate only, leaving the terminal phosphate moiety free which is not possible in the case of CuTPP species. The mechanism of hydrolysis of CuTPP species can also be represented by Fig. 1, the only difference being the absence of proton on β-phosphate.

In view of the above evidence and the information regarding $\Delta S^{\ddagger}$ values and sites of coordination, hydrolysis in the presence of Cu(II) can be visualised as an SN_2 nucleophilic attack of a polar water molecule on the terminal phosphate moiety of TPP in line with the observations made on ATP hydrolysis [2].

In order to understand the structure-reactivity relationship it is of interest to compare the kinetic behaviour of TPP in the presence of Cu(II) with that of ATP, ADP [2, 10] CDP and CTP [1]. TPP shows the same behaviour in the presence of Cu(II) as ADP and ATP but CDP and CTP are rather stable towards hydrolysis. This difference in the kinetic behaviour and the high reactivity of ADP and ATP was attributed to macrochelate formation involving the nucleic base and phosphate chain in metal binding. Therefore, the catalysis of TPP hydrolysis by Cu(II) ions can be rationalised on the basis of macrochelate formation involving both the thiamine moiety and the pyrophosphate chain. This conclusion, made on the basis of the reactivity, supports the structure proposed earlier by potentiometric [2] and other techniques [12].

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