Catalytic Hydrolysis of *p*-Nitrophenyl Picolinate by Copper(II) and Zinc(II) Complexes of N-(2-Deoxy-β-D-glucopyranosyl-2-salicylaldimino)

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ABSTRACT: D-glucosamine Schiff base N-(2-deoxy-β-D-glucopyranosyl-2-salicylaldimino) and its Cu(II) and Zn(II) complexes were synthesized and characterized. The hydrolysis of *p*-nitrophenyl picolinate (PNPP) catalyzed by ligand and complexes was investigated kinetically by observing the rates of the release of *p*-nitrophenol in the aqueous buffers at 25°C and different pHs. The scheme for reaction acting mode involving a ternary complex composed of ligand, metal ion, and substrate was established and the reaction mechanisms were discussed by metal–hydroxyl and Lewis acid mechanisms. The experimental results indicated that the complexes, especially the Cu(II) complex, efficiently catalyzed the hydrolysis of PNPP. The catalytic reactivity of the Zn(II) complex was much smaller than the Cu(II) complex. The rate constant k_N showing the catalytic reactivity of the Cu(II) complex was determined to be 0.299 s^{-1} (at pH 8.02) in the buffer. The pK_a of hydroxyl group of the ternary complex was determined to be 7.86 for the Cu(II) complex. © 2002 Wiley Periodicals, Inc. Int J Chem Kinet 34: 345–350, 2002

INTRODUCTION

Metal-ion-complex-catalyzed reactions of carboxylic acid derivatives have been extensively investigated in recent years as model reactions of metalloenzymes [1–4] such as carboxypeptidase A [5], carbonic anhydrase [6], and related enzymes. It is now well known that the roles of a metal ion are to activate the substrate as an electrophilic catalyst, stabilizing the negative charges that are formed during the reactions, and to act as a source of hydroxide ion at neutral or mild base buffer [7–10]. In related metalloenzymes, the metal ion appears to activate the seine hydroxyl group [11]. Thus it is important for model studies to examine the

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cooperation between hydroxyl groups and metal ion in catalysis.

Most models studied so far involve a ligand and one or two hydroxyl groups through complexation with metal ion for catalytic hydrolysis of the title compound [7,8]. Few examples report the model involving the ligand and multihydroxyl groups (such as sugar) through complexation with metal ions, which can provide more nucleophiles for attacking the substrate. On the other hand, it had been reported, with a few examples, that the complexes of sugar derivants with metal ions are very important in developing new metal-affinity chromatographic material and chiral homogeneous catalysts [12]. In this paper, the effect of the complexes of D-glucosamine with Cu(II) and Zn(II) ions on the hydrolysis of *p*-nitrophenyl picolinate (PNPP) are investigated, the scheme for acting mode established, and the reaction mechanism discussed. The results indicate that the complexes greatly catalyzed the hydrolysis of PNPP.

MATHEMATICAL MODEL INVOLVING METAL-ION-COMPLEX-CATALYZED REACTION

For enzyme catalytic mechanisms, a ternary complex (composed of ligand, metal ion, and substrate) which has particularly low energy during the catalytic reaction is suggested. The process of metal-ion-complex-catalyzed hydrolysis of PNPP is described as follows: A metal complex [ML_n] forms a ternary complex [ML_nS] with a substrate [S] with an association constant K_S, and then an intracomplex nucleophilic substitution reaction takes place in the ternary complex in the rate-limiting step, with a first-order rate constant k_N to afford the products P. The products are also produced through spontaneous hydrolysis, without involving a ternary complex [13]. The process can be written as

$$ML_n + S \overset{K_S}{\Leftrightarrow} ML_nS \overset{K_N}{\rightarrow} P \tag{1}$$

$$K_S = \frac{[ML_nS]}{[ML_n][S]} = \frac{[ML_nS]}{([ML_n]_T - [ML_nS])([S]_T - [ML_nS])} \tag{2}$$

$$S \overset{k_0}{\rightarrow} P \tag{3}$$

where k_0 is the first-order rate constant for hydrolysis of PNPP in buffer. $[S]_T$ and $[ML_n]_T$ are the total concentrations of the substrate and complex respectively.

From the above scheme, it can be expressed that

$$\text{Rate} = k_\psi [S]_T = k_N[ML_nS] + k_0([S]_T - [ML_nS]) \tag{4}$$

where k_ψ is the apparent rate constant for hydrolysis of substrate.

So from Eq. (2), we obtain

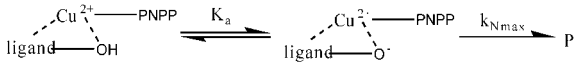
$$[ML_nS] = K_S[ML_n]_T[S]_T/(1 + K_S[ML_n]_T) \tag{5}$$

Inserting Eq. 5 into Eq. 4 and rearranging, we obtain

$$\frac{1}{k_\psi - k_0} = \frac{1}{k_N - k_0} + \frac{1}{(k_N - k_0)K_S}\frac{1}{[ML_n]_T} \tag{6}$$

From Eq. (6), it can be seen that the slope and the intercept of the plot $\frac{1}{k_\psi - k_0}$ vs. $\frac{1}{[ML_n]_T}$ afford the k_N and K_S values.

Suppose the acid dissociation equilibrium takes place in the ternary complex in the buffers to $CuSG{\cdot}2H_2O$.



where k_{Nmax} is the first-order rate constant for product because of the discomposing of ternary complex, and K_a is the acid dissociate constant of ternary complex.

If the dissociation equilibrium is reached rapidly, then the reaction rate is determined by the amount of dissociated complex anion so that we have

$$k_N = \frac{K_a}{[H]^+ + K_a} k_{Nmax} \tag{7}$$

Rearranging Eq. (7), we obtain

$$\frac{1}{k_N} = \frac{1}{k_{Nmax}} + \frac{1}{k_{Nmax}K_a} \cdot [H^+] \tag{8}$$

According to Eq. (8), the k_{Nmax} and k_a values can be obtained from a plot of $1/k_N$ vs. $[H^+]$.

EXPERIMENTAL SECTION

General Methods

Infrared spectra were obtained on a PE 983 IFS FIOIR spectrophotometer. All elementary analyses were performed on an EA1110 elementary analyzer. Kinetic studies were carried out by UV–vis methods, with a GBC 916 UV–vis spectrophotometer equipped with a thermostatic cell holder. The contents of metal

ion in complexes were demarcated by EDTA standard solution.

Materials

All reagents, unless otherwise indicated, were of analytical grade and were used without further purification. Glucosamine hydrochloride was of biochemical grade and was used without further purification. Salicylaldehyde was purified by distillation. *p*-Nitrophenyl picolinate (PNPP) was supplied by the Organic Chemical Laboratory of Sichuan University [14]. PNPP stock solution for kinetics was prepared in acetonitrile.

The water used for syntheses and kinetics was obtained by distilling deionized water twice. To avoid the influence of the chemical component of different buffers, Tris–TrisH^+ buffer was used in all cases and its pH was adjusted by adding analytical pure nitron acid in all runs.

N-(2-Deoxy-β-D-glucopyranosyl-2-salicylaldimino)

(SG·H_2O). Glucosamine hydrochloride (10 mmol) and sodium bicarbonate (12 mmol) were dissolved in 10 ml of water, and salicylaldehyde (10 ml) was added. The mixture was stirred vigorously at room temperature and in 10 min the separation of crystals commenced. After 3 h of stirring, the bright precipitate was filtered, washed with cold water, and dried in vacuum [15]. The compound was found to melt sharply at 183°C.

Anal. Calcd for $C_{13}H_{17}O_6N$: C, 55.12; H, 6.01; O, 33.92; N, 4.94; Found: C, 54.94; H, 6.18; O, 34.01; N, 4.86.

CuSG·2H_2O. A solution of $Cu(Ac)_2$ (10 mmol) in 20 ml methanol was added dropwise to a solution of ligand (10 mmol) in 20 ml of methanol at 45°C. After 2 h of vigorous stirring, the solution was reduced to 10 ml through distillation. The grayish-green precipitate that appeared after the addition of 20 ml of ether was filtered and washed with ether. The complex was dried overnight in vacuum [16].

Anal. Calcd for $CuC_{13}H_{19}O_8N$: C, 40.83; H, 4.97; O, 33.51; N, 3.66; Cu, 17.01; Found: C, 41.58; H, 4.85; O, 33.58; N, 3.53; Cu, 16.45. IR: γ_{OH}, 3423 cm^{-1}; $\gamma_{CH=N}$, 1628 cm^{-1}; γ_{Cu-N}, 372 cm^{-1}; γ_{Cu-O}, 225 cm^{-1}.

ZnSG·H_2O. A solution of $Zn(Ac)_2$ (10 mmol) in 20 ml methanol was added dropwise to a solution of ligand (10 mmol) in 20 ml of methanol at 45°C. After 2 h of vigorous stirring, the solution was reduced to 10 ml through distillation. The light-yellow precipitate that appeared after the addition of 20 ml of ether was filtered and washed with ether. The complex was dried overnight in vacuum.

Anal. Calcd for $ZnC_{26}H_{34}O_{13}N_2$: C, 48.22; H, 5.25; O, 32.15; N, 4.33; Zn, 10.05. Found: C, 48.10; H, 5.35; O, 32.46; N, 4.26; Zn, 9.83. IR: γ_{OH}, 3352 cm^{-1}; $\gamma_{CH=N}$, 1619 cm^{-1}; γ_{Zn-N}, 345 cm^{-1}; γ_{Zn-O}, 226 cm^{-1}.

The structures of ligand and the Cu(II) and Zn(II) complexes are shown in Fig. 1. The ligand and the complexes were all water-soluble in the buffer.

Kinetics Studies

Solutions were prepared in the buffer. Reaction temperature was maintained at 25°C. Release of *P*-nitrophenol was monitored at 400 nm. Each kinetic run was initiated by injecting 15 μl of PNPP (10^{-3} mol dm^{-3} in CH_3CN) into the cuvette containing 3 ml of the buffer solution. Rate constants were obtained from the (linear) plots of $\log(A - A_t)$ vs. time. Each pseudo-first-order rate constant is the mean value of five determinations; its average relative standard deviation is smaller than 1.5%.

RESULTS AND DISCUSSION

The Apparent Rate Constants of the Hydrolysis of PNPP at pH 7.21 and 25°C

The ligand and the complexes are water-soluble and so the kinetics were carried out in plain buffer for all reagents. Table I shows the apparent rate constants

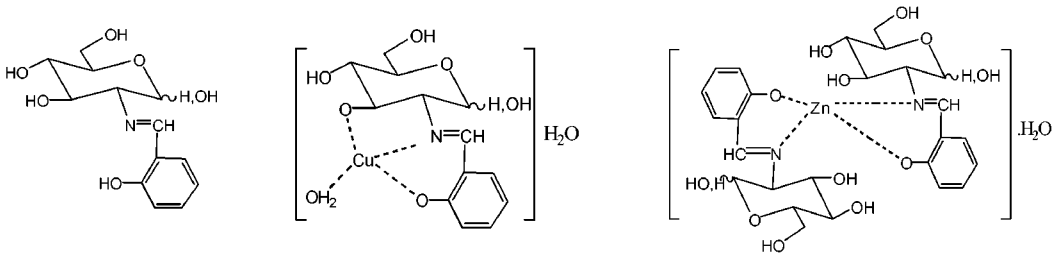


Figure 1 The structures of ligand and Cu(II) and Zn(II) complexes.

Table I Apparent Rate Constants of the Hydrolysis of PNPP at pH 7.21 and 25°C

No.	System	k_ψ (10^{-3} s^{-1})
1	None	0.0575
2	Cu^{2+}	5.566
3	Zn	0.383
4	$SG{\cdot}H_2O$	0.944
5	$CuSG{\cdot}H_2O$	19.75
6	$ZnSG{\cdot}H_2O$	1.271

All data were measured in 10^{-2} mol dm^{-3} Tris–$TrisH^+$ ($I = 0.2$, KCl) buffer at pH 7.21 and 25°C. The concentrations of all reagents are 10^{-4} mol dm^{-3}. [PNPP] $= 5 \times 10^{-5}$ mol dm^{-3}.

(k_ψ) obtained under the condition of an excess reagent over the substrate at pH 7.21 and 25°C. The table indicates that the ligand and complexes were reactive in the hydrolysis of PNPP. The rate enhancement by ligand alone is large, which is more than 18-fold of k_0. It is one of the phenomena in works of complexes imitating hydrolytic metalloenzymes. The reactivity of the ligand to PNPP is probably relative to the space structure of the ligand. The hydroxyls on the glucosamine act as nucleophiles, attacking the substrate. So, though without metal ions, the ligand still can catalyze the hydrolysis of PNPP. The large rate enhancement of more than 400-fold is obtained by the addition of 10^{-4} mol dm^{-3} $CuSG{\cdot}2H_2O$. Even a more remarkable rate enhancement was achieved with Cu(II) or Zn(II) alone, but the catalytic abilities of complexes were much more higher than metal ions alone. This suggests that the formation of a ternary complex accelerated the leaving of *p*-nitrophenol in the aqueous buffer.

Determination of k_N, K_S, and K_a Values

As shown in Fig. 2, the plots of $1/(k_\psi - k_0)$ vs. $1/[ML_n]_T$ is a straight line. From those straight lines,

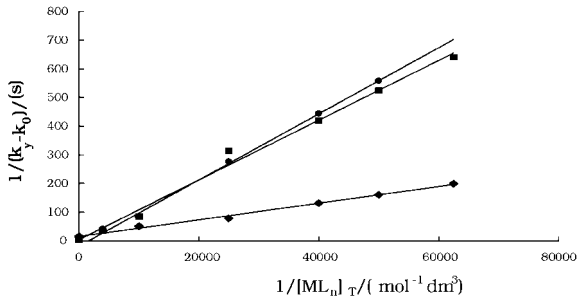


Figure 2 Plots of $CuSG{\cdot}2H_2O$ $1/(k_\psi - k_0)$ vs. $1/[ML_n]_T$ for the hydrolysis of PNPP in 0.01 mol dm^{-3} Tris at 25°C and at different values of pH: ♦, 7.21; ●, 8.02; ■, 7.60, respectively.

Table II pH Dependencies of k_N and K_S in Buffer at 25°C for $CuSG{\cdot}2H_2O$

pH	k_N (10^{-3} s^{-1})	K_S (10^3 mol^{-1} dm^3)
7.21	43.96	8.142
7.60	58.89	1.474
7.75	87.69	0.985
7.90	105.8	0.598
8.02	298.6	0.321

All data were measured in 10^{-2} mol dm^{-3} Tris–$TrisH^+$ ($I = 0.2$, KCl) buffer. [PNPP] $= 5 \times 10^{-5}$ mol dm^{-3}.

it can be seen that the ternary complex mathematical model proposed is reasonable, and the value of k_N and K_S could be obtained from those straight lines.

Tables II and III list the rate constants and dissociation constants, respectively, for complexes at 25°C and different pHs. $CuSG{\cdot}2H_2O$ is the most active complex in reagents and the k_N of it is to be 0.299 s^{-1} at 25°C and pH 8.02, which is more than 5200-fold of k_0. With the different pH values, the plot of $1/k_N$vs. $[H^+]$ is shown in Fig. 3.

According to Eq. (8), the pK_a for the ternary complex to $CuSG{\cdot}2H_2O$ was obtained to be 7.86. The pK_a decreases from 14 to 7.86, meaning that the metal-bound water molecule ionizes and yields a high fraction of hydroxide at mild base buffer. Such a metal-bound hydroxide ion is a potent nucleophile retaining most of the reactivity of free hydroxide ions. The observed stoichiometry was 1:1 for $CuSG{\cdot}2H_2O$ and the proposed reaction mechanism is shown in Fig. 4. In a ternary complex, the complex of PNPP with Cu^{2+} involving pyridine nitrogen is very important at the rate-limiting step of attacking a ligand-oxide anion nucleophile on the substrate carbonyl carbon to form an additional intermediate [17,18]. The intermediate was then disclosed, the product was released, and the reaction restarted. In principle,

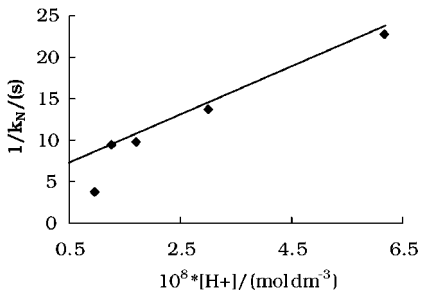


Figure 3 Plot of $1/k_N$ vs. $[H^+]$ for the reaction of PNPP with $CuSG{\cdot}2H_2O$ in 0.01 M Tris.

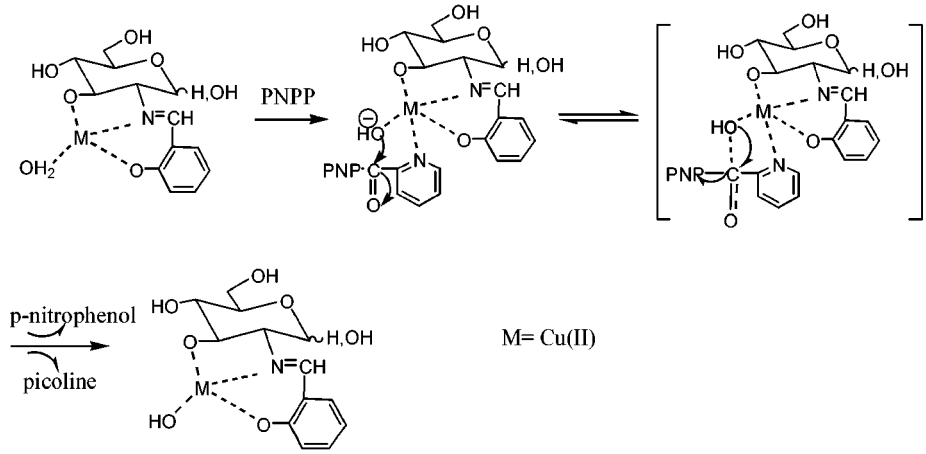


Figure 4 Proposed mechanism of $CuSG{\cdot}2H_2O$ catalysis of hydrolysis of PNPP in 0.01 mol dm^{-3} Tris.

$CuSG{\cdot}2H_2O$ could catalyze the hydrolysis of PNPP by the metal hydroxide mechanism [10]. The important roles of Cu(II) ion in the buffer is to activate the substrate and produce hydroxide ions acting as nucleophiles.

As we know, hydrolytic metalloenzymes in nature generally bind their substrates and then use the action of two or more well-placed functional groups to achieve catalysis. The observed stoichiometry is 1:2 for $ZnSG{\cdot}H_2O$. In Fig. 1, it is shown that Zn(II) complexes have no empty space for coordinating with PNPP, and it's well known that the ability of Zn(II) to activate water molecules to be nucleophile is much smaller than Cu(II) [18], and so the activity of $ZnSG{\cdot}H_2O$ was smaller than that of $CuSG{\cdot}2H_2O$. But the k_N of $ZnSG{\cdot}H_2O$ is 85-fold of the rate constant of the PNPP divided spontaneously at pH 8.02 and 25°C in the buffer. The important reason why $ZnSG{\cdot}H_2O$ could catalyze the hydrolysis of PNPP probably is the hydroxides of glucosamine acting as nucleophiles to attack carbonyl carbon of substrate to form an acylation intermediate, as shown in Fig. 5. Then the acylation intermediate released acyl, and the reaction was finished and restarted. So, the k_N of $ZnSG{\cdot}H_2O$ also can be looked as the constant of deacylation. This proposed catalytic mechanism was similar to those of cyclodextrins [19–22]. Because hydroxide is hydrophilic, the ligand and complexes are easily soluble in water. In aqueous solution, the complex depended on hydrogen bonds and van der Waals forces between substrate and catalyst to hold the substrate molecule tightly, and then the nucleophile attacked the carbonyl carbon of PNPP. In some means, this behavior of complexes imitates integration position of hydrolytic metalloenzyme. This is very important in designing artificial enzymes. The function of Zn(II) in the intermediate is to stabilize the structure of ternary complex and stabilize the negative charges.

In summary, the Cu(II) and Zn(II) complexes of *N*-(2-deoxy-β-D-glucopyranosyl-2-salicylaldimino) are good catalysts for hydrolysis of PNPP in the plain buffer at 25°C. Although Cu^{2+} and Zn^{2+} had catalytic reactivity to the hydrolysis of PNPP, their complexes had higher activity than metal ions alone, and the catalytic ability of $CuSG{\cdot}2H_2O$ is much larger than that of $ZnSG{\cdot}H_2O$. So multihydroxyl metal complexes have extensive prospects in the field of artificial hydrolytic metalloenzymes.

Table III pH Dependencies of k_N and K_S in Buffer at 25°C for $ZnSG{\cdot}H_2O$

pH	k (10^{-3} s^{-1})	K_S (10^3 mol^{-1} dm^3)
7.10	1.155	3.851
7.21	3.145	2.374
7.60	17.63	2.403
8.02	36.74	2.613

All data were measured in 10^{-2} mol dm^{-3} Tris–$TrisH^+$ ($I = 0.2$, KCl) buffer. [PNPP] = 5×10^{-5} mol dm^{-3}.

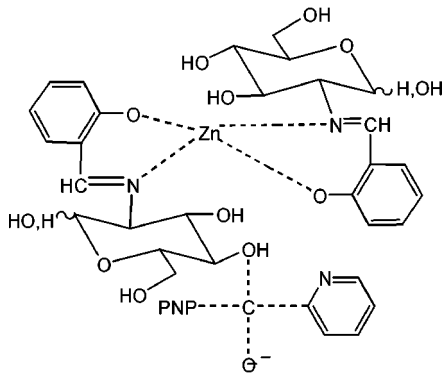


Figure 5 Proposed mechanism of $ZnSG{\cdot}H_2O$ catalysis of hydrolysis of PNPP in 0.01 mol dm^{-3} Tris.

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