第9期 2002年9月 无机化学学报 CHINESE JOURNAL OF INORGANIC CHEMISTRY Vol. 18, No. 9 Sep., 2002

# 大环钴(Ⅱ)配合物模拟水解酶催化羧酸酯水解的比较研究

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在Brij35胶束溶液中,比较研究了四氮大环席夫碱(5,7,7,12,14,14-六甲基-1,4,8,11-四氮杂十四环-二烯,L)的钴(Ⅱ)配合物1催化对硝基苯酚吡啶甲酸酯(PNPP)及对硝基苯酚乙酸酯(PNPA)水解的动力学。结果表明:配合物1对PNPP及PNPA的催化作用具有酸碱催化的特征,催化活性物种为与金属离子结合的氢氧根离子CoL-OH⁻;配合物1催化PNPP水解的速度远远大于其催化PNPA水解的速度,在pH 7.40、30℃时,表观二级速率常数 $k_c$ 分别为0.997 $mol^{-1} \cdot L \cdot s^{-1}$ 和 $1.12 \times 10^{-3} mol^{-1} \cdot L \cdot s^{-1}$,这种反应速率的差异可归因于反应机理的不同;Brij35胶束对PNPP及PNPA的水解均有抑制作用。

关键词: 钴(Ⅱ)配合物 羧酸酯 水解 催化动力学

分类号: O614.81⁺2

# Comparative Study on Catalytic Cleavage of Carboxylic Esters using Macrocyclic Co (Ⅲ) Complex as Imitator of Hydrolase

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The comparative kinetic investigation of the hydrolysis of p-nitrophenyl picolinate(PNPP) and p-nitrophenyl acetate(PNPA) catalyzed by the tetracoordinate macrocyclic Schiff base complex of Co (Ⅲ) (**1**) in Brij35 micellar system at 30℃ is reported. The results indicate that **1** catalyzed hydrolysis of PNPP and PNPA is acid-base catalytic process and the active species is metal bound hydroxide ion, i. e., CoL-OH⁻. **1** promoted hydrolysis of PNPP proceeds much faster than that of PNPA. At pH 7.40, the apparent second-order rate constants kc for hydrolysis of PNPP and PNPA are 0.997 $mol^{-1} \cdot L \cdot s^{-1}$ and $1.12 \times 10^{-3} mol^{-1} \cdot L \cdot s^{-1}$, respectively. The difference of hydrolytic rate may be attributed to the difference of hydrolytic mechanisms by which the PNPP and PNPA operate. The results also reveal that the hydrolysis of PNPP and PNPA are inhibited by Brij35 micelle. The reasons are discussed in detail.

**Keywords:** **cobalt (Ⅲ) complex** **carboxylic esters** **hydrolysis** **catalytic kinetics**

The roles of metal ions in hydrolytic metalloenzymes have been a topic of considerable interest. Among the enzymes that have been mimicked in enzyme model studies, the Zn (Ⅱ) -containing hydrolytic metalloenzyme carboxypeptidase A (CPA) has been well studied[1]. The functions of metal ion in this enzyme are considered[2]: (a) to activate water molecule as nucleophile at neutral pH; (b) to serve as a template

收稿日期:2002-04-22。收修改稿日期:2002-06-18。

国家自然科学基金资助项目(No. 20173038)。

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to bring substrate and nucleophile together in a ternary complex; (c) to activate the carbonyl of the substrate. Substitution of Co (Ⅱ) for Zn (Ⅱ) of CPA results in an even more active enzyme[3]. This interchangeability of zinc and cobalt was also found in other hydrolytic enzymes[4,5].

The divalent metal ions catalyzed hydrolytic cleavage of esters in micellar systems have been extensively studied as the models of hydrolytic metalloenzymes[6-8]. Tagaki et al[9]studied systematically the hydrolysis of PNPP catalyzed by the metal ion complexes of lipophilic derivatives of imidazole co-micelled with surfactants. Scrimin et al[10] and we[11, 12] investigated the reactivity of a series of hydrophobic pyridines toward PNPP in the presence of Cu (Ⅱ) and Zn (Ⅱ) in micellar systems. All of above works demonstrated that the critical feature of the catalytic process is the formation of a ternary complex involving ligand, metal ion and substrate, in which the hydroxyl properly bound to a ligand may be activated by metal ion and acts as effective nucleophile in a pseudo-intramolecular process. However, most of micellar models for hydrolytic metalloenzymes involved Cu (Ⅱ) or Zn (Ⅱ) ion complexes, while Co (Ⅱ) ion complexes were seldom employed[13, 14] as biomimetic models for hydrolytic metalloenzymes.

The fact that Zn (Ⅱ) in zinc-containing metalloenzymes can be replaced in most case by Co (Ⅱ) in vitro without losing catalytic activity[15] or with resulting in more active enzyme in some case inspires us to consider that Co (Ⅱ) complex of the tetracoordinate macrocyclic Schiff base ligand can be an effective catalyst for hydrolysis of carboxylic esters. Therefore, in this paper, we investigated the hydrolysis of carboxylic esters catalyzed by macrocyclic Schiff base complex of Co (Ⅱ) in Brij35 micellar solution.

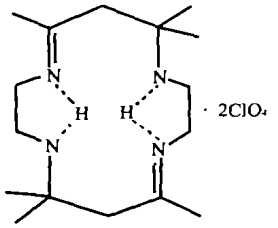
## 1 Experimental

### 1.1 Materials

Brij35($C_{12}H_{25}(OCH_2CH_2)_{23}OH$) is sigma product and was used as received. Tris(trihydroxylmethyaminomethane), $HNO_3$, $KNO_3$, acetonitrile are of analytical reagent grade. PNPA was obtained from tokyo kasei kogyo Co. and used without further purification. PNPP was prepared and purified by literature method[16]. The water used for kinetic experiment was obtained by distilling deionized water. The stock solutions of PNPA and PNPP were prepared in acetonitrile.

### 1.2 Synthesis

Macrocyclic Schiff base ligand, 5, 7, 7, 12, 14, 14-hexamethyl-1, 4, 8, 11-tetraazacycl-otetradeca-4, 11-dienedihydrogen perchlorate, $C_{16}H_{32}N_4 \cdot 2HClO_4$ ($L \cdot 2HClO_4$, Scheme I): It was prepared according to the literature[17]. Anal. Calcd. For $C_{16}H_{34}Cl_2N_4O_8$: C, 39.92; H, 7.12; N, 11.64%. Found: C, 40.15; H, 7.21; N, 11.70%.




Scheme I

$[Co(C_{16}H_{32}N_4)](ClO_4)_2$ ($CoL \cdot 2ClO_4$, **1**): This complex was prepared primarily according to the literature[18]. It was found that the yield is low. Therefore the method similar to that employed in the literature[19] was applied to the preparation of the complex. Experimental results indicated that the composition of the product is same as that of product obtained according to the literature[18] but the yield is much higher. Anal. Calcd. For $C_{16}H_{32}N_4O_8Cl_2Co$: C, 35.70; H, 5.99; N, 10.41%. Found: C, 35.61; H, 5.95; N, 10.28%.

### 1.3 Determination

A pHS-3A pH meter was used for the pH determination and control. Elemental analysis was performed on MOD 1106 elemental analyzer. Kinetic runs were conducted by using a GBC 916 spectrophotometer equipped with a thermostatic cell compartment. Reaction temperature was maintained at 30℃. Reaction was initiated by injecting 30μL of acetonitrile solution of substrate into a 1-cm cuvette containing 3mL of desired concentration of complex in micellar solution. Pseudo-first-order rate constants for the hydrolysis of substrate ester were determined by monitoring the release of *p*-nitrophenolate at 400nm under the conditions of excess of catalyst over substrate. Reactions were generally followed for at least 6 half-lives. Pseudo-first-

order rate constants were obtained by initial rate method for PNPA and by using $\ln(A_\infty - A_t) - \ln(A_\infty - A_0) = -k_{obsd}t$ for PNPP. Kinetic runs carried out in triplicate gave rate constants with uncertainty of less than 3%.

## 2 Results and Discussion

### 2.1 General Survey of Rate for Hydrolysis of PNPP and PNPA in Different Systems

The pseudo-first-order rate constants ($k_{obsd}$) of different systems are shown in Table 1. It indicates that the complex **1** can accelerate the hydrolysis of both PNPP and PNPA either in plain buffer or in buffered Brij35 micelle. But the rate enhancements for hydrolysis of PNPP (by a factor of about 160 both in micellar and non-micellar solution) are much larger than those for hydrolysis of PNPA(by a factor of 2.2 in plain buffer and 1.1 in buffered micelle respectively), indicating that the different mechanisms may be involved for hydrolysis of PNPP and PNPA (*vide infra*).

The data in Table 1 also show that both the pseudo-first-order rate constant for **1** catalyzed hydrolysis and that for spontaneous hydrolysis in Brij35 micellar solution are smaller than those in plain buffer, indicating that the inhibition for hydrolysis of PNPP and PNPA occurs in non-ionic surfactant Brij35 micellar solution. The reasons may be ascribed to the following: (a) the hydrophobic substrates are incorporated into the micellar phase, while the water-soluble metallocomplexes are distributed over the bulk and the micellar phase[13]; (b) the twined long polyoxyethylene chain hinders the movement of molecules of the reactants[20]; (c) the greater availability of water in non-micellar system may result in a greater hydration of the complex **1**[14]. These results indicate that the Brij35 micellar microenvironment is not favorable for hydrolysis of carboxylic esters.

### 2.2 Kinetic Investigation of Hydrolysis of PNPP and PNPA in Brij35 Micellar Solution

Fig. 1 shows the variation of the pseudo-first-order rate constants($k_{obsd}$) for hydrolysis of PNPP and PNPA, respectively, as a function of **1** concentration at different pHs. From Fig. 1, it can be seen that the $k_{obsd}$ increases linearly with increasing **1** concentration. No saturation kinetics is observed, indicating that the binding of substrates to **1** is weak[13].

The apparent second-order rate constant $k_c$ can be derived from the slop of the plot of the $k_{obsd}$ vs the concentration of **1**[21]. The results obtained were listed in Table 2.

Table 2 shows that $k_c$ increases with the increase of pH. This indicates that **1** promoted hydrolysis of PNPP and PNPA is an acid-base catalytic process and the active species is the dissociated complex, i.e. CoL-$OH^-$ species. Furthermore, the plots of $k_c$ vs pH give two sigmoidal curves, as shown in Fig. 2, which is characteristic feature of acid-base catalysis. This also suggest that the active species in hydrolysis is CoL-$OH^-$ species, as proposed by Kimura et al[21] for macrocyclic polyamine complex catalyzed esters hydrolysis and acetaldehyde hydration.

But on the basis of the structure of complex **1**[18],

**Table 1 Pseudo-First-Order Rate Constants for Hydrolysis of PNPP and PNPA in Different Systems***

| No | system | $10^3 k_{obsd}$(PNPP)/$s^{-1}$ | $10^5 k_{obsd}$(PNPA)/$s^{-1}$ |
|---|---|---|---|
| 1 | buffer | 0.0442 | 1.82 |
| 2 | buffer + **1** | 7.09 | 4.03 |
| 3 | buffer + Brij35 | 0.026 | 1.11 |
| 4 | buffer + Brij35 + **1** | 4.35 | 1.27 |

*: In 0.1mol · $L^{-1}$ Tris-$HNO_3$ buffer, $\mu$ = 0.1($KNO_3$); [Brij35] = $5.00 \times 10^{-3}$mol · $L^{-1}$; [**1**] = $6.40 \times 10^{-3}$mol · $L^{-1}$; [PNPP] = [PNPA] = $5.00 \times 10^{-5}$mol · $L^{-1}$; pH = 7.15; $T$ = 30℃.

**Table 2 Apparent Second-Order Rate Constants for 1 Promoted Hydrolysis of PNPP and PNPA***

| substrate | pH | $k_c$/(mol$^{-1}$ · L · s$^{-1}$) | substrate | pH | $10^3 k_c$/(mol$^{-1}$ · L · s$^{-1}$) |
|---|---|---|---|---|---|
| PNPP | 7.15 | 0.595 | PNPA | 7.15 | 0.457 |
| | 7.40 | 0.997 | | 7.40 | 1.12 |
| | 7.80 | 2.31 | | 7.80 | 2.52 |
| | 8.09 | 2.48 | | 8.09 | 2.94 |

*: [PNPP] = [PNPA] = $5.00 \times 10^{-5}$mol · $L^{-1}$, [Brij35] = $5.00 \times 10^{-3}$mol · $L^{-1}$, $\mu$ = 0.1($KNO_3$); $T$ = 30℃.

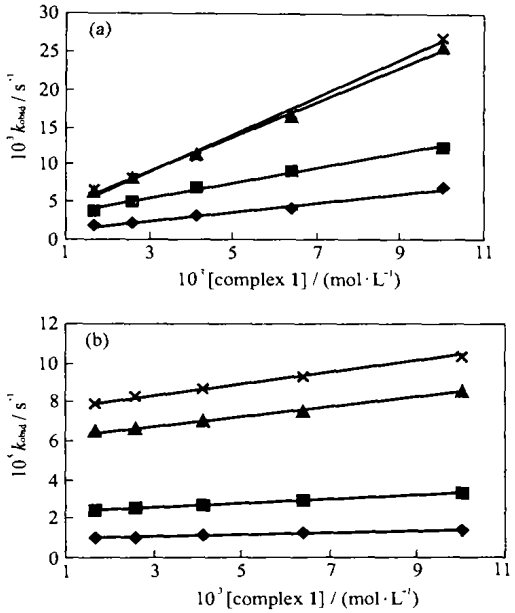



Fig. 1　Plots of $k_{obsd}$ vs **1** concentration for hydrolysis of PNPP(a) and PNPA(b)
$I$ = 0.1($KNO_3$), [Brij35] = 5.00 × 10$^{-3}$mol · L$^{-1}$, [PNPP] = [PNPA] = 5.00 × 10$^{-5}$mol · L$^{-1}$, pH = 7.15 (◆), 7.40(■), 7.80(▲). 8.09(×).

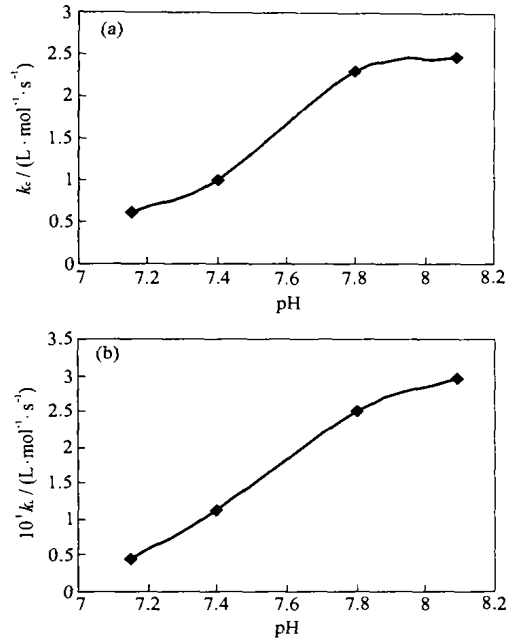



Fig. 2　Plots of second-order rate constant ($k_c$) vs pH for **1** promoted hydrolysis of PNPP(a) and PNPA (b)

no water molecule is found to coordinate to Co (Ⅱ). Therefore, the pre-equilibrium of hydration of the complex **1** may exist before the formation of tetrahedral intermediate. That is, one water molecule may be bound to Co (Ⅱ) to form hydrated complex CoL-$H_2O$ in solution. The deprotonation of CoL-$H_2O$ gives the catalytically active species CoL-$OH^-$.

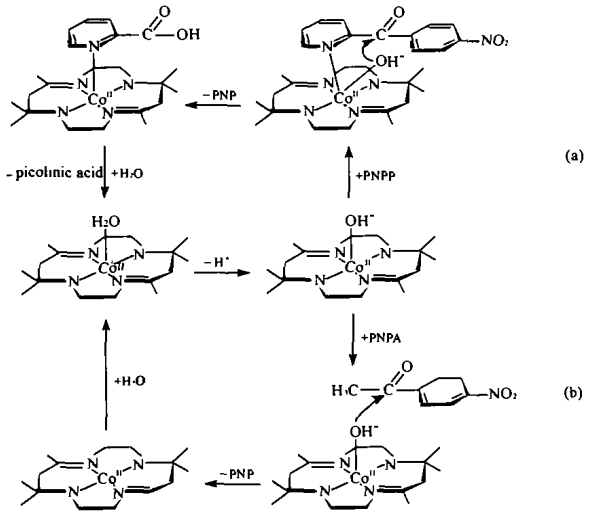
$$\text{complex } \mathbf{1} + H_2O \rightleftharpoons \text{CoL-}H_2O \overset{k_a}{\rightleftharpoons} \text{CoL-}OH^- + H^+ \quad (\mathbf{1})$$

The deprotonation constant p$k_a$ of CoL-$H_2O$ can be estimated from the pH value at which the inflection of Sigmoidal curve occurred. Fig. 2 shows that the inflection points of two sigmoidal curves are almost at the same pH(7.6). Thus the estimate of p$k_a$ of CoL-$H_2O$ is about 7.6, which is 2.1 units lower than that of the water in hydrated cobalt ion, $Co(H_2O)_6^{2+}$[22]. This suggests that the concentration of the metal bound hydroxide ion CoL-$OH^-$ which is a effective nucleophile[23, 24], is much higher than that of hydroxide ion under neutral or mildly alkaline conditions. Consequently, the active species for **1** promoted hydrolysis of PNPP and PNPA is the metal bound hydroxide ion CoL-$OH^-$ other than hydroxide ion.

From Figs. 1 and 2, it can be seen that the same active species CoL-$OH^-$ shows different catalytic activities toward hydrolysis of PNPP and PNPA. The rate for hydrolysis of PNPP is much faster than that for PNPA, suggesting that the hydrolysis of PNPP and PNPA may proceed via different mechanisms. Since the PNPP is a metallophilic substrate, it may form a reactive ternary complex[16] with CoL-$OH^-$ by the coordination of picolinate nitrogen to cobalt (Ⅱ). Therefore the mechanism for **1** promoted hydrolysis of PNPP may be that cobalt (Ⅱ) in CoL-$OH^-$ keeps holding the substrate, and then $OH^-$ in CoL-$OH^-$ species attacks the substrate to carry out the intramolecular nucleophilic reactions, as shown in Scheme II (a). Breslow and co-workers[25] has proposed the analogous mechanism for zinc-catalyzed hydrolysis of anhydrides.

As widely accepted, in enzymatic catalysis enzymes generally bind their substrates and then use the action of two or more well-placed functional groups synergistically to achieve catalysis[26]. This makes enzymatic reactions become intramolecular reactions which is often proceed much faster than their intermolecular counterparts[27].

Compared to PNPP, PNPA is unable to form any




Scheme II Possible mechanisms for **1** promoted hydrolysis of PNPP (a) and PNPA (b)

ternary complex with **1** because it has no coordinative functional group which can serve as an effective ligand to the metal ion[16] . Thus, the mechanism for **1** catalyzed hydrolysis of PNPA may involve an intermolecular nucleophilic attack of cobalt bound hydroxide ion CoL-$OH^-$ at the carbonyl of PNPA, as shown in Scheme II(b), which is less effective than intramolecular reaction mechanism[27] for **1** promoted catalysis of PNPP. As stated above, **1** catalyzed hydrolysis of PNPA may be an intermolecular nucleophilic reaction, which differs entirely from the hydrolytic process catalyzed by natural hydrolytic metalloenzyme. Therefore, it is reasonable that the hydrolytic reaction of PNPA catalyzed by **1** proceeds much slower than that of PNPP.

## 3 Conclusion

The present investigation clearly shows that the macrocyclic Schiff base complex of cobalt (Ⅱ) can function as a effective synzyme for hydrolysis of PNPP and PNPA. It exhibits a much higher catalytic activity toward hydrolysis of PNPP than PNPA. The catalytic activity of macrocyclic Schiff base complex of cobalt (Ⅱ) in Brij35 micellar system is lower than that in plain buffer. Thus, the macrocyclic Schiff base complex of cobalt (Ⅱ) in plain buffer system is a better potential model for hydrolytic metalloenzyme than that in Brij35 micellar system.

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