Hydrolytic activity of μ -alkoxide/acetato-bridged binuclear Cu(II) complexes towards carboxylic acid ester

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Abstract. Two μ -alkoxide/acetate-bridged small molecule binuclear copper(II) complexes were synthesized, and used to promote the hydrolysis of a classic carboxylic acid ester, *p*-nitrophenyl picolinate (PNPP). Both binuclear complexes exhibited good hydrolytic reactivity, giving rise to *ca.* 15547- and 17462-fold acceleration over background value for PNPP hydrolysis at neutral conditions, respectively. For comparing, activities of the other two mononuclear analogues were evaluated, revealing that binuclear complexes show approximately 150- and 171-fold kinetic advantage over their mononuclear analogues.

Keywords. Bimetallic synergic effect; hydrolysis; carboxylic acid ester; kinetics.

1. Introduction

Design and synthesis of artificial hydrolases towards esterolysis received considerable attention due to its projected roles in some fields.^{1–3} One of the vital important aspects is investigating the structure–activity relationship of artificial hydrolases, which are able to facilitate the development of novel and efficient mimic enzymes. In particular, binuclear complexes have been widely focused because this type of complexes probably mimics the binuclear motif of native enzymes with high activity. At present, useful information was obtained in many studies^{4–7} concerning impacts of various factors (catalyst structure, pH, substrate concentration, temperature, etc.) on hydrolysis of esters, and some good catalysts have been developed. As for *p*-nitrophenyl picolinate (PNPP), to our knowledge, its hydrolytic acceleration by synthesized catalysts was not up to four orders of magnitude under neutral conditions. Therefore, we report the best result for the hydrolytic acceleration of PNPP by two μ -alkoxide/acetato-bridged binuclear copper(II) complexes (figure 1) with two hydroxyl-functional Salen-type ligands (H₃L1 and H₃L2), achieving remarkable rate enhancement by a factor of excess four orders of magnitude. To examine if binuclear complexes indeed

possess much better reactivity, moreover, two mononuclear analogues with the same Schiff base compounds as references were applied to promote PNPP hydrolysis.

2. Experimental

Schiff base ligands (H₃L1 and H₃L2) were synthesized by reacting salicylaldehyde (or 5-chlorosalicylaldehyde) with 1, 3-diamino-2-propanol. Corresponding binuclear complexes were prepared by a similar procedure,⁸ and mononuclear analogues were synthesized according to earlier studies.⁹ Related characterizations (Notes) determined the structures of these synthesized complexes. Hydrolytic kinetics of PNPP was monitored by the release of *p*-nitrophenolate anion at 400 nm (see the north-facing arrow in figure 2 denoting increasing absorbance of *p*-nitrophenolate anion) on GBC Cintra 1010 UV-vis spectrometer (Australia) on the basis of initial rate method.¹⁰

3. Results and discussion

In general, metal-induced hydrolysis of PNPP includes an intramolecular nucleophilic attack of metal-bound hydroxide or H₂O on the carboxyl of substrate linked with central metal.^{11–13} In our cases, the coordinated acetate molecule in Cu₂L would not remain coordinated and expected to be replaced by water molecule(s) under

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Figure 1. Chemical structures of ligand (H_3L), binuclear copper(II) complexes (Cu_2L) and mononuclear copper(II) complexes (CuL).

Figure 2. UV spectra of Cu_2L1 -catalysed hydrolysis of PNPP in buffered solution. The insert represents the nucleophilic attack of the metal-bound hydroxide on the carboxyl of PNPP in the substrate–catalyst binary complex.

the current experimental conditions. In figure 2, profiles for $PNPP+Cu_2L1$ spectra indicate that substrate (PNPP) actually links to complex molecule. Similar spectra for the catalytic system containing Cu_2L2 are not shown here. Two possible binding routes (route *a* or *b* in the insert of figure 2) involve in the course of formation of $PNPP-Cu_2L$ intermediates by the replacement of one water molecule bound to one copper centre with PNPP. The coordination of substrate molecule to the catalytic centre is generally regarded as a key step for inducing transformation of an intermolecular route to an intramolecular one,¹⁴ which contributes to the decrease in reaction activation energy besides creating a high effective concentration resulting in high reaction rates. Further, another Cu(II)-bound hydroxide group as a nucleophilic reagent attacks the carboxyl of Cu(II)-bound PNPP releasing the detectable *p*-nitrophenolate anion. Last, one free water molecule

displaces the Cu(II)-bound picoline acid, liberating catalyst molecules.

In the current study, hydrolysis of PNPP by both binuclear copper complexes gives acceleration blindly at almost neutral environment. Pseudo-first order rate constant (k_{ob}) for the Cu_2L1 -promoted PNPP hydrolysis was found to be 0.121 s^{-1} , representing 15547-fold rate increase in comparison with the uncatalysed reaction¹⁵ ($k_{uncat} = 7.8 \times 10^{-6}\text{ s}^{-1}$) at pH 7.0. It is note worthy that Cu_2L2 containing 5'-chloride substituent shows about 1.12-fold kinetic advantage over its analogue (Cu_2L1) under compared conditions. Namely, Cu_2L2 contributed the hydrolysis of PNPP with a 17462-fold acceleration. In the two systems that were used, the huge acceleration probably results from bimetallic synergistic effects (activating substrate as well as lowering pK_a of the water bound to central copper).

Most of the natural enzymes exhibit optimum activity under physiological conditions.¹⁶ Conversely, the inactivation of enzymes occurs due to denaturation of enzymes under some extreme conditions (e.g., stronger acidity–alkalinity, high temperature).¹⁷ Hence, developing artificial enzymes with high efficiency under near neutral conditions is a big challenge. In profiles of k_{ob} -pH (figure 3) it appears that the PNPP hydrolytic cleavage displays distorted bell-shaped curves, which is in accordance with the typical characters of most of the natural enzymes. Optimum hydrolysis rates reached at pH 7.30 for both catalytic systems in the presence of binuclear complexes, suggests that Cu(II)-induced hydrolysis of PNPP is indeed pH-dependent and the Cu(II)-bound water molecule is deprotonated to produce a real nucleophilic Cu_2L -hydroxide species. Estimated pK_{a1} (~ 7.1) of the copper(II)-bound H_2O inside Cu_2L2 by the inflection points of k_{ob} -pH curve is relatively lower than that (~ 7.2) of Cu_2L1 -bound H_2O , providing one of the evidences for better

Figure 3. Variations of apparent rate constant (k_{ob}) with pH for the PNPP hydrolysis by Cu₂L1(a) and Cu₂L2(b) in the pH range of 6.5–8.5. Conditions: 298 ± 0.1 K, $I = 0.1$ mol L⁻¹ (KCl), [catalyst] = 1.0×10^{-5} mol·L⁻¹. [PNPP]: $\blacklozenge$, 0.200 mmol L⁻¹; $\blacksquare$, 0.267 mmol L⁻¹; $\blacktriangle$, 0.333 mmol L⁻¹; $\diamond$, 0.400 mmol L⁻¹; $\circ$, 0.467 mM. At a constant 0.200 mM PNPP concentration, distinguishing reactivity of binuclear copper(II) complexes and their mononuclear analogues (CuL1 and CuL2) towards the hydrolysis of PNPP (0.200 mmol L⁻¹) was evaluated (each insert in figure 3), respectively.

reactivity of the former due to more convenient formation of active Cu₂L2(H₂O)OH at the same pH. An electron-withdrawing effect of 5'-substituted chloride gives a positive driving force for lowering pK_{a1} of the bound water for Cu₂L2, arousing slightly higher activity of Cu₂L2. Both pK_{a1} values for the two systems are in neutral pH range, which confirms that the binuclear Cu(II) complexes are able to obviously lower pK_a of the Cu(II)-bound water. Also, we observed that half-life ($t_{1/2}$) of Cu₂L2-induced PNPP hydrolysis is about 8.5 s as well as 10.4 s for the catalytic system containing Cu₂L1 under conditions of pH 7.30, [PNPP] = 0.467 mmol·L⁻¹, [Cu₂L] = 1.0×10^{-5} mol·L⁻¹. PNPP of a given concentration was almost completely hydrolysed at 3 min intervals. The obtained half-lives testify that binuclear complexes used in this case are highly efficient.

The pH dependence of the obtained catalysis constant (k_{cat}) is shown in figure 4. The k_{cat} for Cu₂L2-catalysed hydrolysis of PNPP was calculated as 5.61×10^3 s⁻¹, while a relatively smaller k_{cat} for Cu₂L1 was given as 3.01×10^3 s⁻¹ at optimum pH (7.30). As shown in figure 4, we are inclined to believe that the rate-limiting step of the hydrolysis of PNPP is similar to the changing tendency of k_{ob} as a function of pH. In addition, a sharp increasing and declining of k_{cat} near pH 7.3 indicates that both catalysts reach corresponding preponderant reactivity only in a highly narrow pH zone. Low or high pH values lead to decreasing activity by the two complexes, which is probably a result of the generation of dehydrated species with smaller nucleophilicity or inactive di-hydroxide species.

It is noteworthy that the difference (approximately 0.1 pK unit) in pK_{a1} value is not so much for the two binuclear complexes. However, k_{cat} values still exhibit

Figure 4. Dependence of the catalytic constant (k_{cat}) with pH for the PNPP hydrolysis catalysed by Cu₂L. Conditions: 298 ± 0.1 K, $I = 0.1$ mol L⁻¹ (KCl), [catalyst] = 1.0×10^{-5} mol·L⁻¹.

observable difference by a factor of about 1.9-fold even if this difference is not remarkable. This fact indicates that improvement in activity of Cu₂L2 is mainly caused by reduction of electron density on the target carbonyl carbon through coordination bond between the nitrogen atom (route *a* in figure 2) or carbonyl oxygen (route *b* in figure 2) of substrate (PNPP) and one of two copper(II) centra. Lewis acidity of the copper(II) of Cu₂L2 is stronger than that Cu₂L1 as result of electron-withdrawing effect of 5'-substituted chloride of Cu₂L2, resulting in better nucleophilic reactivity of carbonyl group of PNPP bound to Cu₂L2 than that bound to Cu₂L1. Additionally, experimental observations showed that mononuclear complexes displayed much lower reactivity than their bimetallic analogues

(refer to inserts in figure 3) under comparable conditions, suggesting approximately 150- and 171-fold difference in hydrolysis rate. On one hand, a *cis*-hydroxide position is the most desirable for the metal-catalysed hydrolysis reaction. This *cis*-orientation relationship between copper(II)-bound hydroxide and PNPP is not easy to maintain and control in the case of PNPP hydrolysis by mononuclear CuL ($\text{L} = \text{L1}$ or L2), leading to smaller hydrolysis acceleration of PNPP. On the other hand, both mononuclear copper(II) complexes in this case show classic sigmoid $k_{\text{ob}}\text{-pH}$ curves, giving bigger $\text{p}K_{\text{a1}}$ values (about 8.0) against their binuclear analogues with a $\text{p}K_{\text{a1}}$ of ~ 7.0 . As for the two catalytic systems in the presence of mononuclear complexes, the real active CuL-OH species are considered to be maintained at a lower level of concentration in the pH region used under near neutral buffered solutions. Ultimately, small rate enhancement was observed in comparison with uncatalysed reaction. In contrast, the two binuclear complexes can provide the binding site as well as double Lewis acid activation^{18–21}, and therefore display higher activity for catalysing hydrolysis of PNPP.

Apart from the effect of pH on the PNPP hydrolysis, we also investigated the temperature effect in our case (figure 5). Normally, higher temperature causes more collisions among the molecules and more collisions increase the likelihood that substrate will collide with the active site of the enzyme, thus increasing the rate of an enzyme-catalysed reaction. Above a certain temperature, however, enzyme activity begins to decline because of the temperature-induced denaturation^{22,23}. It was found that both complexes used in our study have distorted and well-known bell-shaped curves, that is, $\text{Cu}_2\text{L1}$ reached its best activity at 308 K; whereas

$\text{Cu}_2\text{L2}$ showed own optimum efficiency at 328 K. This result reveals that reactivity of the two synthesized complexes is temperature-dependent. At the same time, this bell-shaped changing tendency probably originated from the thermo-allosteoric behaviour of the present complexes in the range of 298–338 K. At 308 K and 328 K, in other words, the two copper(II) complexes were probably given fitted conformations that facilitate the coordination of substrate (PNPP) to the active site *via* a route similar as an ‘induced-fit’ theory²⁴ of natural enzymes.

4. Conclusion

In summary, this study reports the noteworthy acceleration (excess 4-order acceleration of magnitude) of the hydrolysis of *p*-nitrophenyl picolinate by two synthesized bimetallic complexes in neutral solution. The two synthetic complexes called artificial enzymes display enzyme-like catalytic performances, e.g., high activity, mild optimum pH, physiological temperature and bell-shaped dependence of rate upon temperature. Small acceleration in mononuclear complex-catalysed hydrolysis of PNPP demonstrates that binuclear complexes generally possess much higher reactivity compared to their mononuclear analogues. Hence, we can predict that both binuclear complexes mentioned above probably show much better activity in the micellar solutions owing to the positive effects of micelle aggregates on the hydrolytic process. In addition, the reactivity of the two complexes will be evaluated towards the hydrolytic cleavage of ester bonds in other phosphate or carboxylic esters besides PNPP. Related studies by our group are in progress.

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Notes

Characterizations of synthesized ligands, mono- and binuclear copper(II) complexes are shown as follows.

(i) $\text{H}_3\text{L1}$: Yellow solid, yield 56.3%, m.p. 102.5–103.2°C. ^1H NMR(400 MHz, CDCl_3): $\delta(\text{ppm})=$

Figure 5. Varying of K_{ob} versus temperature for Cu_2L -induced PNPP hydrolysis in the zone of 298–338 K. Conditions: $I = 0.1 \text{ mol}\cdot\text{L}^{-1}$ (KCl), $[\text{catalyst}] = 1.0 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, $[\text{PNPP}] = 0.200 \text{ mmol}\cdot\text{L}^{-1}$.

13.15(s, 2H, ArOH, exchanged proton in D₂O), 8.43(s, 2H, N = CH), 6.88–7.35(m, 8H, ArH), 4.27–4.30(m, 1H, CH–CH₂), 3.72–3.91(m, 4H, NCH₂), 2.12(s, 1H, CH–OH); IR(KBr, film) ν_{max} : 3394, 3055, 2896, 1633, 1579, 1496, 1459, 1277, 1206, 1037, 753; Anal. calcd. for C₁₇H₁₈N₂O₃: C 68.44, H 6.08, N 9.39%; found C 69.01, H 6.04, N 9.44%.

(ii) *H₃L2*: Yellow solid, yield 85.5%, m.p. 108.4–109.3°C. ¹H NMR(400 MHz, CDCl₃): δ (ppm) = 13.05(s, 2H, ArOH, exchanged proton in D₂O), 8.43(s, 2H, N = CH), 6.92–7.54(m, 6H, ArH), 4.30–4.31(m, 1H, CH–CH₂), 3.71–3.92(m, 4H, NCH₂), 2.04(s, 1H, CH–OH); IR(KBr, film) ν_{max} : 3426, 3124, 2889, 1636, 1512, 1480, 1273, 1163, 827; Anal. calcd. for C₁₇H₁₆N₂Cl₂O₃: C 55.60, H 4.39, N 7.63%; found C 55.72, H 4.46, N 7.82%.

(iii) *Cu₂L1*: Dark green solid, yield 78.08%, m.p. > 216.9°C (dec.). *m/z*: calc. for C₁₉H₁₈Cu₂N₂O₅: 479.98; found 480.9(M+H), 502.9(M+Na). Anal. calcd. for C₁₉H₁₈Cu₂N₂O₅: C 47.4, H 3.77, N 5.82%; found C 47.72, H 3.75, N 5.78%.

(iv) *Cu₂L2*: Dark green solid, yield 67.82%, m.p. > 202.5°C (dec.). *m/z*: calc. for C₁₉H₁₆Cu₂Cl₂N₂O₅: 549.9; found: 551.5(M+H), 573.0(M+Na). Anal. calcd. for C₁₉H₁₆Cu₂Cl₂N₂O₅: C 41.47, H 2.93, N 5.09%; found C 41.43, H 3.10, N 5.02%.

(v) *CuL1*: Dark green solid, yield 88.63%, m.p. > 260°C. *m/z*: calc. for C₁₇H₁₆CuN₂O₃: 359.05; found: 360.1(M+H), 382.3(M+Na). Anal. calcd. for C₁₇H₁₆CuN₂O₃: C 56.74, H 4.48, N 7.78%; found C 57.37, H 4.42, N 7.84%.

(vi) *CuL2*: Yellow-green solid, yield 91.01%, m.p. > 260°C. *m/z*: calc. for C₁₇H₁₄CuCl₂N₂O₃: 426.97; found: 428.0(M+H), 450.06(M+Na). Anal. calcd. for C₁₇H₁₄CuCl₂N₂O₃: C 47.62, H 3.29, N 6.53%; found C 48.45, H 3.41, N 6.61%.

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