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CATALYSIS OF PHOSPHODIESTER TRANSESTERIFICATION BY CU(II)-TERPYRIDINE COMPLEXES WITH PERIPHERAL PENDENT BASE GROUPS: IMPLICATIONS FOR THE MECHANISM

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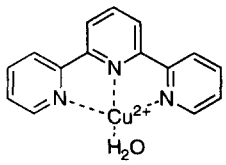
Abstract: In this paper we show that in phosphodiester transesterification catalyzed by copper(II)-terpyridine complexes both the metal-bound hydroxyl group and the peripheral pendent tertiary amino groups can provide general base catalysis. At neutral pH the pendent amino groups dominate the process because the metal-bound hydroxyl group is protonated. However, the metal-bound hydroxyl group is more effective at high pH, probably due to its higher basicity and/or closer location to the bound substrate.

Copper(II)-terpyridine complex **1** is among the most active transition metal complexes that catalyze the hydrolysis of RNA.[1-2] However, **1** and other artificial nucleases are still several orders of magnitude less



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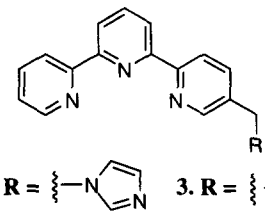

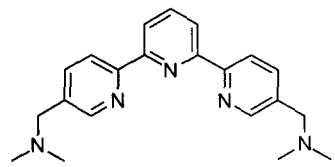

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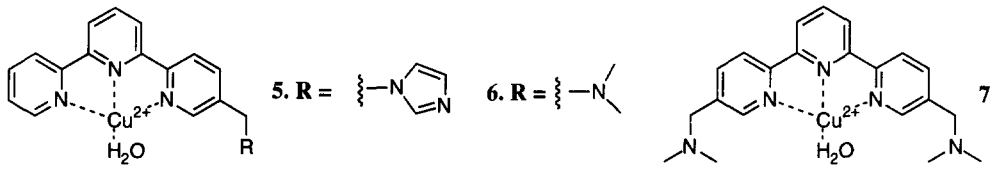
active than natural ribonucleases. Therefore, there is much interest in developing artificial nucleases with higher activities. Since it is well established that general base catalysis is required for the hydrolysis of RNA[3] and model compounds,[4-5] we have prepared terpyridine derivatives **2-4** and their corresponding Cu(II) complexes **5-7**. The rationale behind our design is that the base group(s) on the periphery of **5-7** may increase the activity of the complexes by providing general base catalysis.[6] In this communication we compare the activities of **5-7** with **1** in catalyzing the transesterification reaction of 2-hydroxypropyl-4-nitrophenyl phosphate (HPNPP), a model compound for RNA.[7]

Ligands **2** and **3** were prepared from 5-bromomethyl-2:2',6':2"-terpyridine[8] by reaction with imidazole and dimethylamine, respectively. Difuctionalized ligand **4** was similarly formed from 5, 5"-dibromomethyl-2:2',6':2"-terpyridine and dimethylamine.[8,9] Complexes **5-7** were freshly generated from the corresponding chlorides which were obtained from evaporation of equimolar solutions of Cu(II)Cl_2 and **2-4** in methanol. Complex **1** was prepared according to the method of Chin.[2] Kinetic measurements for the catalysis of the transesterification reaction of HPNPP by **1** and **5-7** began with preparation of the stock solutions of the catalysts in buffer solutions at the desired pH. The catalyst solutions were filtered through a 0.5 μm microfilter unit and pipetted (1900 μL) into UV cuvettes. The cuvettes were placed in the sample compartment of a Hewlett-Packard UV spectrophotometer and equilibriated for 20 minutes. The reaction was



initiated by the addition of an aliquot (100 μL) of a freshly prepared solution of HPNPP in pure water. The reaction was followed by monitoring the increase of absorbance at 400 nm. The pseudo first-order rate constants (k_{obs}) were calculated from the initial slopes of absorbance-time traces, the concentrations of the substrate and the extinction coefficients of p-nitrophenoxide ion in the reaction media. The first-order rate constants (k_{uncat}) for background hydrolysis were obtained similarly. The values of k_{obs} and k_{uncat} are reproducible with less than 5% error.

Table 1. The corrected pseudo first-order rate constants (k_{obs} - k_{uncat}) for hydrolysis of HPNPP in pH 7.0 HEPES buffer (0.05 M) at 25 °C in the presence of Cu(II) complexes.[a]

catalyst	**1**	**1**[b]	**5**	**6**	**7**
k_{obs} - k_{uncat} (s^{-1})	5.74×10^{-6}	2.36×10^{-6}	6.96×10^{-7}	1.53×10^{-5}	3.89×10^{-5}

[a][HPNPP] = 2.0×10^{-4} M, [Cu(II) complex] = 2.0×10^{-3} M, $k_{uncat} = 1.13\times10^{-7}$ s^{-1}. [b][Imidazole] = 2.0×10^{-3} M.

Table 1 summarizes the corrected pseudo first-order rate constants (k_{obs} - k_{uncat}) for hydrolysis of HPNPP catalyzed by Cu(II) complexes **1** and **5**-**7**. The peripheral tertiary amino groups increase the activities of **6** and **7** by nearly 3- and 7-fold, respectively, as compared with **1**. However, the imidazole group on the periphery of **5** decreases its activity by more than 8-fold relative to **1**. This is surprising, because previous studies have shown that a pendent imidazole group increased the ribonuclease activities of Zn(II) complexes.[5,10] One possible explanation may be that the pendent imidazole group in **5** causes dimerization, as illustrated in Figure 1.[10] Such intermolecular coordination will lower the activity of **5** because the imidazole group occupies the metal coordination site that is used by water or the substrate (see discussion below). This explanation is supported by the observation that addition of imidazole to a solution of **1** also diminished its activity (Table 1). Apparently, the tertiary amino groups in **6** and **7** do not bind to the Cu(II) ion, probably due to steric hindrance. However, the rate enhancements afforded by the peripheral tertiary amino groups are modest. To find out if this is due to protonation of the amino groups in the neutral reaction medium, pH-rate profiles were obtained for **1**, **6** and **7** (Figure 2). Figure 3 shows the dependence of the ratio of k_{obs} - k_{uncat} for **6** and **7** to that for **1** on the pH of the reaction medium. The rate enhancements afforded by the peripheral tertiary amino groups decrease with increase of pH and vanish at high pH. For example, **7** is more than 15 times more active than **1** at pH 6.5 but the two catalysts have almost identical activities at pH

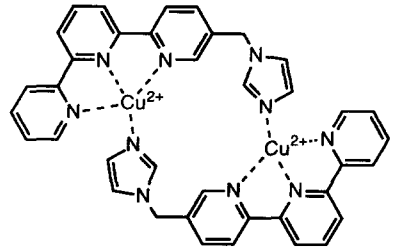


Figure 1. Intermolecular Coordination of the peripheral imidazole group in **5**

9.5. An explanation is available from examination of the pH-rate profiles for **1**, **6** and **7** in Figure 2. From the profile for **1** it is evident that a base group with pK_a around 8 is essential for catalysis. This should be a Cu(II)-bound hydroxide group.[2] The pH-rate profiles for **6** and **7** indicate that there is an additional base group with a pK_a around 6.5 involved in catalysis. This base group should be the peripheral tertiary amino residue in **6** and **7**. Apparently, at high pH the Cu(II)-bound hydroxide group is much more effective than the

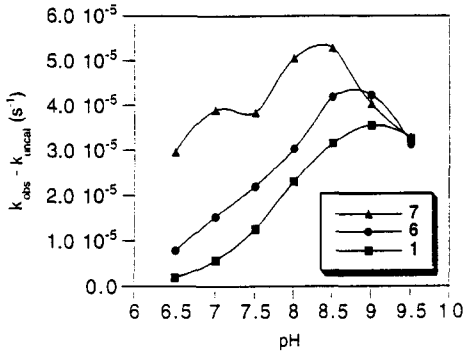


Figure 2. The pH-rate profiles for transesterification of HPNPP catalyzed by **1**, **6** and **7**.

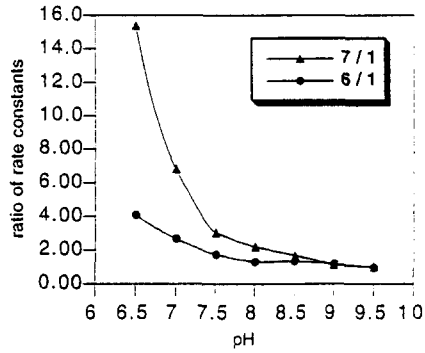


Figure 3. Comparison of activities of **1**, **6** and **7** at different pH.

Conditions for Figures 2 and 3: [HPNPP] = 2.0×10^{-4} M; [catalyst] = 2.0×10^{-3} M. Temperature: 25 °C. The reaction media with pH 7.0, 7.5 and 8.0 are HEPES buffers (0.05M); those with pH 8.5, 9.0 and 9.5 are AMPSO buffers (0.05M); the one with pH 6.5 is MES buffer (0.05M).

peripheral tertiary amino group in providing general base catalysis, propably due to closer location and higher basicity. As a result, **1**, **6** and **7** have the same activity at high pH. But the peripheral tertiary amino group is the major provider of general base catalysis at low pH because most of the Cu(II)-bound hydroxide groups are now protonated. This is why at low pH, **6** and **7** are more active than **1** and the activity increases with the number of the peripheral tertiary amino groups. Previous studies[2,11] have emphasized the importance of Lewis acid activation in explaining the ribonuclease activities of Cu(II) complexes. The results obtained in the present study demonstrate that the presence of the Cu(II)-bound hydroxide group is also essential for high activity. Therefore, an effective substrate-catalyst complex should also include at least one Cu(II)-bound hydroxide group. For catalyst **1** this requires that the Cu(II) ion is at least pentacoordinated, as illustrated in Figure 4. It was suggested above that imidazole inhibits the activity of **1** by occupying a coordination site of the Cu(II) ion. This is more easily understood with the mechanism shown in Figure 4: replacement of either the hydroxide group or the substrate by imidazole should result in either diminished or lost activity for **1**.

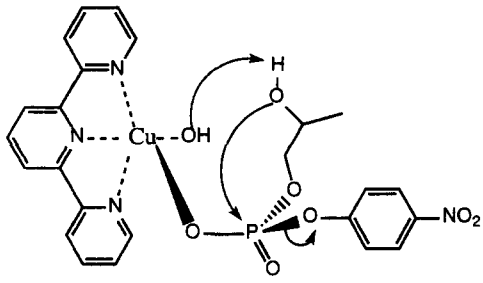


Figure 4. Proposed mechanism for catalysis of the transeterification of HPNPP by **1**

In summary, we have demonstrated that the Cu(II)-bound hydroxide group is essential for the ribonuclease activity of the Cu(II)-terpyridine complex **1**, but its effectiveness is limited by its protonation in near neutral media. In these solutions the activity of **1** can be improved by attaching tertiary amino groups on the periphery of the complex.

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(9) All new compounds gave satisfactory spectroscopic data. Compound **2** (white solid, mp. 188-189 °C): ^{1}H NMR ($CDCl_3$) δ 8.70 (d, 1H), 8.59 (m, 3H), 8.45 (t, 2H), 7.97 (t, 1H), 7.86 (t, 1H), 7.63 (s, 1H), 7.60 (m, 1H), 7.36 (m, 1H), 7.14 (s, 1H), 6.95 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 156.4, 155.9, 155.9, 155.4, 149.1, 147.9, 137.9, 137.3, 136.8, 135.8, 131.7, 130.2, 123.8, 121.2, 121.1, 120.0, 119.0, 48.2. Anal. calcd. for $C_{19}H_{14}N_5$: C, 72.83; H, 4.82; N, 22.35. Found: C, 72.55; H, 4.93; N, 22.24. **3** (white solid, mp. 81-82 °C): ^{1}H NMR ($CDCl_3$) δ 8.70 (d, 1H), 8.58 (m, 3H), 8.44 (d, 1H), 7.96 (t, 1H), 7.85 (m, 2H), 7.31 (m, 1H), 3.52 (s, 2H), 2.29 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 156.2, 155.3, 149.7, 149.1, 137.9, 137.6, 136.9, 134.4, 123.8, 121.2, 120.9, 120.8, 61.3, 45.3. Anal. calcd. for $C_{18}H_{18}N_4$: C, 74.46; H, 6.25; N, 19.30. Found: C, 74.31; H, 6.28; N, 19.27. **4** (off-white solid, mp. 96-97 °C): ^{1}H NMR ($CDCl_3$) δ 8.60 (s, 2H), 8.58 (d, 2H), 8.43 (d, 2H), 7.94 (t, 1H), 7.83 (m, 2H), 3.52 (s, 4H), 2.29 (s, 12H); ^{13}C NMR ($CDCl_3$) δ 155.3, 149.7, 137.9, 137.6, 134.3, 120.9, 120, 8, 61.3, 45.3. HRMS m/e for $C_{21}H_{25}N_5$ calcd 347.2110, obsd 347.2108.

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