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Metallocyclodextrin catalysts for hydrolysis of phosphate triesters

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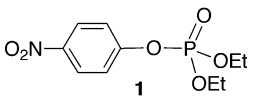
Abstract—The copper complexes of 6^A-(2-aminoethylamino)- and 6^A-(3-aminopropylamino)-6^A-deoxy-β-cyclodextrin catalyse the hydrolysis of 4-*tert*-butyl-2-nitrophenyl dimethyl phosphate, with $k_{inc} = 3.1\times10^{-2}$ and 2.3×10^{-2} s^{-1}, and $K_d = 4.3\times10^{-4}$ and 1.2×10^{-3} M, respectively, in 0.05 M pH 7.0 HEPES buffer at 298 K. This corresponds to rate accelerations of more than 95 000 and 70 000 times for reaction of the cyclodextrin-bound species.

Cyclodextrins have been studied extensively as catalysts and enzyme mimics.[1] Much of this work has been focussed on reactions of carboxylate esters[2] but relatively little has been concerned with the hydrolysis of phosphate triesters. Natural[3] and modified[4] cyclodextrins are known to either inhibit, or only modestly accelerate, breakdown of these organophosphates in aqueous solutions. Even when reaction occurs, in some cases the cyclodextrins are thereby phosphorylated, so they are not acting as true catalysts. Organophosphates such as paraoxon **1** are important as insecticides and nerve agents.[5] There is considerable interest in methods for the catalytic breakdown of these compounds, for the bioremediation of contaminated soil and water, and the destruction of stockpiles of chemical warfare agents.[6] Therefore, we set out to develop cyclodextrin-based catalysts for hydrolysis of phosphate triesters.



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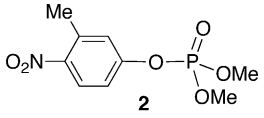

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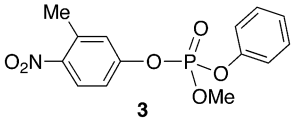

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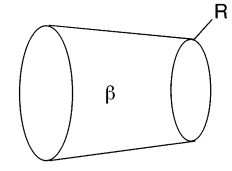
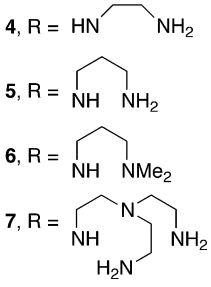
Various metal complexes are known to catalyse the reactions of phosphate triesters.[7–9] For example, the Cu(II) complex of *N*,*N*,*N*′,*N*′-tetramethyl-1,2-diaminoethane increases the rates of hydrolysis of paraoxon **1** and the related compounds **2** and **3** by 2–3 orders of magnitude.[9] Accordingly, in our studies we employed the Cu(II) complexes of the modified β-cyclodextrins **4-7**,[10] to exploit the catalytic properties of the metal and the binding characteristics of the cyclodextrins. The principal substrate chosen for this investigation was the triester **8**, in which the *tert*-butyl group on the aromatic ring promotes complexation by the cyclodextrin[11] and the nitro group facilitates detection of the hydrolysis product **9** (Scheme 1).

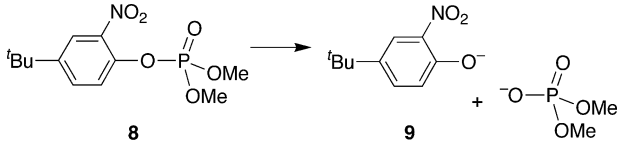


R = OH, β-cyclodextrin



4, R = HN(CH2)2NH2
5, R = NH(CH2)3NH2
6, R = NH(CH2)3NMe2
7, R = N(CH2CH2NH2)(CH2)3NH2

The organophosphate **8** was produced by phosphorylation of 4-*tert*-butylphenol with dimethyl phosphorochloridate,[12] followed by nitration.[13] Hydrolysis of this compound was monitored at 435 nm, which corre-



8 **9**

Scheme 1.

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sponds to the λ_{max} of the phenoxide **9** ($\varepsilon = 3700$ M^{-1} cm^{-1}). At this wavelength, the triester **8** shows negligible absorption. At 298 K, in 0.05 M HEPES buffer containing 1% methanol, at pH 8.0 and 7.0, and in 0.05 M MES buffer containing 1% methanol, at pH 6.0, hydrolysis of a 3×10^{-4} M solution of the triester **8** occurred in a pseudo-first order manner, with rate constants (k_{un}) of 2.3×10^{-6}, 3.2×10^{-7} and $<1\times10^{-7}$ s^{-1}, respectively.[14] By comparison, in the presence of the Cu(II) complex of 6^A-(2-aminoethylamino)-6^A-deoxy-β-cyclodextrin **4** (1×10^{-3} M),[15] but under otherwise identical conditions, pseudo-first order rate constants (k_{obs}) of 4.6×10^{-2}, 2.9×10^{-2} and 3.4×10^{-3} s^{-1} were observed for the hydrolysis, corresponding to rate increases of ca. 20 000, 91 000 and >34 000, respectively. For the Cu(II) complex of 6^A-(3-aminopropylamino)-6^A-deoxy-β-cyclodextrin **5**, the corresponding values for k_{obs} were found to be 2.1×10^{-2}, 9.9×10^{-3} and 1.0×10^{-3} s^{-1}, showing increases in hydrolysis rates of ca. 9000, 31 000 and >10 000. By contrast, the Cu(II) complexes of the cyclodextrins **6** and **7** had much less effect. When used at a concentration of 1×10^{-3} M, at pH 7.0 they increased the rate of hydrolysis of the triester **8** (k_{obs}) by factors of only 2800 and 380, respectively. While the Cu(II) complexes of the cyclodextrins **4** and **5** were found to be effective catalysts, their components were not. At pH 7.0, 1×10^{-3} M solutions of β-cyclodextrin, 6^A-(2-aminoethylamino)-6^A-deoxy-β-cyclodextrin **4**, 6^A-(3-aminopropylamino)-6^A-deoxy-β-cyclodextrin **5**, Cu(II) and the Cu(II) complexes of 1,2-diaminoethane[15] and 1,3-diaminopropane increased the rate of hydrolysis of the triester **8** by factors of only 2, 2, 2, 20, 10 and 40.

When ranges of concentrations of the triester **8** and the Cu(II) complexes of the cyclodextrins **4** and **5** were used, saturation kinetics were observed, as is typical of enzyme-catalysed reactions and characteristic of discrete substrate binding and reaction processes. Under these conditions, the rate constant for reaction of the complexed triester **8** (k_{inc} or k_{cat}) and the equilibrium constant for complexation of the triester **8** by the metallocyclodextrin (K_d or K_m) were determined to be 5.0×10^{-2} s^{-1} and 3.8×10^{-3} M at pH 8.0, and 3.1×10^{-2} s^{-1} and 4.3×10^{-3} M at pH 7.0, with the cyclodextrin **4**, and 4.8×10^{-2} s^{-1} and 1.2×10^{-3} M at pH 8.0, and 2.3×10^{-2} s^{-1} and 1.2×10^{-3} M at pH 7.0, with the cyclodextrin **5**. It was not practical to determine the corresponding values at pH 6.0 for two reasons. Firstly, at this pH the rates of hydrolysis of the phosphate **8** are too slow to measure accurately when only low concentrations of metallocyclodextrin are used. Secondly, the conjugate acid of the phenoxide **9** has a pK_a of 7.48 at 303 K,[16] so only a small percentage of the hydrolysis product is detectable as the chromophoric species **9** at pH 6.0.

Hydrolysis of the triester **8** by the metallocyclodextrins is most probably brought about by metal-bound hydroxide (Fig. 1).[8,9] This is consistent with the pH dependence observed in the k_{obs} and k_{inc} values reported above. Water bound to copper in the complex of the cyclodextrin **5** is reported to have a pK_a of 7.84,[10] and accordingly, with this system k_{obs} increases by almost a factor of ten when the pH is increased from 6.0 to 7.0, but only by a factor slightly above two when the pH is increased from 7.0 to 8.0.

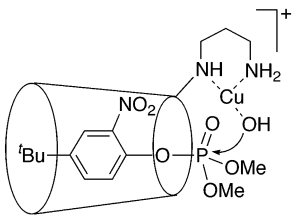
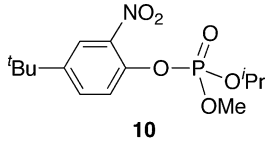


Figure 1. Hydrolysis of the phosphate triester **8** by hydroxide bound to copper in the complex with 6^A-(3-aminopropylamino)-6^A-deoxy-β-cyclodextrin **5**.

Multiple turnover (>10) of the substrate **8** by the copper complex of 6^A-(3-aminopropylamino)-6^A-deoxy-β-cyclodextrin **5** was observed. Thus, the metallocyclodextrin is a true catalyst for the hydrolysis. It was also observed to be substrate selective. For example, it had very little effect on the rate of hydrolysis of paraoxon **1**. It also displayed a low degree of stereoselectivity. The racemate **10** was prepared by sequential treatment of phosphorus oxychloride with 4-*tert*-butylphenol, 2-propanol and methanol, in the presence of triethylamine, followed by nitration.[17] Using the triester **10** (3×10^{-4} M) and the Cu(II)–cyclodextrin **5** complex (either 1.0×10^{-3} or 1.5×10^{-4} M), in 0.05 M HEPES buffer containing 1% methanol at pH 7.0 and 298 K, the rates of hydrolysis (k_{obs}) of the enantiomers differed by a factor of 1.5. Practical limitations prevented an analysis of this stereoselectivity in terms of the separate contributions of binding and reaction of the bound species.



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In summary, the Cu(II) complexes of the cyclodextrins **4** and **5** are effective catalysts for the hydrolysis of the phosphate triester **8**. At pH 7.0 and 298 K, they increase the rate of reaction by more than 95 000 and 70 000 times (k_{inc}/k_{un}), respectively.

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13. *4*-tert-*Butyl-2-nitrophenyl dimethyl phosphate* ***(8)***. 4-*tert*-Butylphenol (0.75 g, 5.0 mmol) was dissolved in toluene (4 mL) and ether (2 mL) cooled to 5–10°C. Sodium hydroxide solution (25%, 3 mL) and dimethyl phosphorochloridate (0.64 mL, 6.0 mmol) were added simultaneously over 30 min. The resulting suspension was stirred for 4 h at room temperature, then poured into ethyl acetate (40 mL). The organic solution was separated and washed with 10% sodium hydroxide solution (2×30 mL) and water (30 mL), then dried ($MgSO_4$) and evaporated under reduced pressure to give an oil (0.5 g). This oil was added slowly to an ice-cooled mixture of HNO_3 (1 mL) and H_2SO_4 (1 mL). The resulting solution was stirred on ice for 12 min, poured into ice-water (20 mL) and extracted with ethyl acetate (2×30 mL). The combined ethyl acetate extracts were washed with brine (2×30 mL) followed by saturated $NaHCO_3$ solution (2×30 mL), dried ($MgSO_4$) and evaporated to give a yellow oil. Chromatography of the oil on silica, eluting with 50% ethyl acetate/hexane, gave the *title compound* ***(8)*** as a pale yellow oil (0.36 g, 24%), which was further purified by bulb to bulb distillation (oven temperature 200°C, 40 mmHg). ^1H NMR ($CDCl_3$): δ 1.25 (s, 9H), 3.82 (d, $J_{P-H}=9.6$ Hz, 6H), 7.46 (d, $J=8.7$ Hz, 1H), 7.62 (dd, $J=8.7$, 2.4 Hz, 1H), 7.92 (d, $J=2.4$ Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 30.8, 34.5, 55.1 (d, $J_{P-C}=6.5$ Hz), 120.2 (d, $J_{P-C}=6.2$ Hz), 121.7 (d, $J_{P-C}=2.6$ Hz), 125.3, 131.2 (d, $J_{P-C}=1.4$ Hz), 140.6 (d, $J_{P-C}=5.7$ Hz), 148.8 (d, $J_{P-C}=1.4$ Hz); EI MS (70 eV) m/z: 303 (M^+, 15%), 288 (100), 257 (65), 227 (10), 163 (10), 127 (23), 109 (21), 77 (8). Anal. calcd for $C_{12}H_{18}NO_6P$: C, 47.53; H, 5.98; N, 4.62. Found: C, 47.25; H, 5.87; N, 4.79.
14. Results obtained from repeating each kinetic experiment were reproducible to within ±10%.
15. The Cu(II) complexes of the cyclodextrins **4**–**7**, 1,2-diaminoethane and 1,3-diaminopropane were prepared in situ using equimolar quantities of Cu(II) perchlorate and amine. Under the conditions used, the Cu(II) is effectively fully complexed since the binary association constants for such complexes are typically $>10^7$ M^{-1}. For example, values of 5.7×10^9 and 6.9×10^7 M^{-1}, have been reported for the cases of 1,3-diaminopropane (du Preez, J. G. H.; van Brecht, B. J. A. M. *J. Chem. Soc., Dalton Trans.* **1989**, 253) and the modified cyclodextrin **5**,[10] respectively.
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17. *4*-tert-*Butyl-2-nitrophenyl isopropyl methyl phosphate* ***(10)***. To a solution of 4-*tert*-butylphenol (3.0 g, 20 mmol) in benzene (10 mL) was added triethylamine (3.06 mL, 22 mmol) followed by phosphorus oxychloride (1.87 mL, 20 mmol). As a precipitate formed, further benzene (20 mL) was added and the mixture was stirred at 90°C for 60 min. 2-Propanol (1.5 mL, 20 mmol) and triethylamine (3.06 mL, 22 mmol) were added and the resulting solution was stirred at 90°C for 60 min. Methanol (0.8 mL, 20 mmol) and triethylamine (3.06 mL, 22 mmol) were then added and the mixture was stirred for a further 60 min at 90°C, then poured into ethyl acetate (150 mL). The solution was washed with aqueous HCl (1N, 3×100 mL), then saturated $NaHCO_3$ (2×100 mL), dried ($MgSO_4$) and evaporated to give an oil (4.78 g). Column chromatography of the oil on silica eluting with a gradient of hexane and ethyl acetate gave an oil (1.89 g). A fraction of the oil (0.25 g) was slowly added to an ice-cooled mixture of HNO_3 (1 mL) and H_2SO_4 (1 mL). The resulting solution was stirred on ice for 12 min, then poured into ice-water (20 mL). The mixture was extracted with ethyl acetate (2×30 mL). The combined ethyl acetate extracts were washed with brine (2×30 mL), followed by saturated $NaHCO_3$ solution (2×30 mL), dried ($MgSO_4$) and evaporated to give a yellow oil. Chromatography of the oil on silica eluting with a gradient of hexane and ethyl acetate gave the *title compound* ***(10)*** as a pale yellow

oil (0.21g, 24%), which was further purified by bulb to bulb distillation (oven temperature 200°C, 40 mmHg). ^{1}H NMR ($CDCl_3$): δ 1.25 (s, 9H), 1.26 (d, $J=5.7$ Hz, 3H), 1.30 (d, $J=6.0$ Hz, 3H), 3.80 (d, $J_{P-H}=11.7$ Hz, 6H), 4.73 (m, 1H), 7.39 (dd, $J=8.7$, 1.2 Hz, 1H), 7.52 (dd, $J=8.7$, 2.4 Hz, 1H), 7.82 (dd, $J=2.4$, 1.2 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 30.9, 34.7, 53.3, 55.1 (d, $J_{P-C}=6.6$ Hz), 74.6 (d, $J_{P-C}=6.3$ Hz), 121.2 (d, $J_{P-C}=2.6$ Hz), 122.2, 131.2, 140.6 (d, $J_{P-C}=6.8$ Hz), 140.8 (d, $J_{P-C}=5.7$ Hz), 148.7; EI MS m/z (70 eV): 331 (M^+, 25%), 316 (50), 290 (43), 274 (82), 243 (100), 180 (70), 105 (15), 91 (19), 77 (22). Anal. calcd for $C_{14}H_{22}NO_6P$: C, 50.76; H, 6.69; N, 4.23. Found: C, 50.74; H, 6.41; N, 4.17. Chiral HPLC analysis of the oil [Chromtech AB Chiral AGP 100×4 mm column, eluting with 7.5% 2-propanol in 0.01 M aqueous phosphate buffer (pH 7.0), flow rate 0.9 mL min^{-1}] showed two enantiomers with retention times of 45.6 and 51.8 min.