Macrocyclic Enzyme Model System: Catalysis of Ester Degradation by a [20]Paracyclophane Bearing Nucleophilic and Metal-binding Sites

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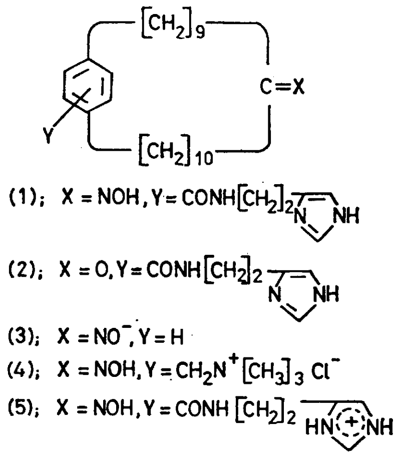
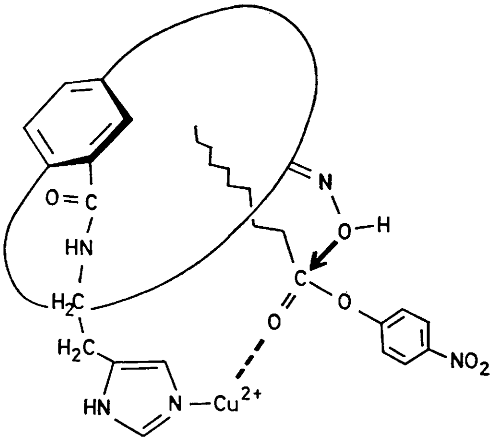
Summary When the [20]paracyclophane (**1**) bearing oxime and imidazole groups is bound to Cu^{2+} ion with the latter species in a moderate pH region, the resulting Cu^{II} complex shows esterase-like activity; the un-ionized oxime group is acylated with the assistance of the co-ordinated Cu^{2+} ion.

PREVIOUSLY, we have reported on the catalytic activity of the [20]paracyclophane (**2**) bearing an imidazole group[1] and noticed a novel aspect of its catalytic function as a result of the introduction of an oxime group and incorporation of Cu^{2+} ion. The reaction of carboxypeptidase A (CPA) has been claimed to proceed through the formation of a mixed anhydride intermediate if a carboxylic ester is employed as a substrate.[2] The suggested mechanism for CPA catalysis involves nucleophilic attack by the γ-carboxylate of glutamate-270 on the substrate carbonyl activated by co-ordination of Zn^{2+} ion. We report here that the bifunctional [20]paracyclophane (**1**)† can be co-ordinated to Cu^{2+} ion through its imidazole group and the catalytic mode of the (**1**)-Cu^{II} complex in ester degradation is similar to the suggested mechanism for CPA [activation of the carbonyl group of the bound substrate by metal co-ordination and the concurrent attack on it by the nucleophile (the un-ionized oxime group)].





(6)

In the absence of Cu^{2+} ion, both (**1**) and (**2**) ($5{\cdot}0 \times 10^{-6}$ mol l^{-1}) effectively deacylated a hydrophobic ester, *p*-nitrophenyl hexadecanoate (PNPP; initial concentration $1{\cdot}0 \times 10^{-5}$ mol l^{-1}); the imidazole group of the para-cyclophanes acts as a nucleophile since the reaction ceases under the present conditions upon complete acylation of the imidazole group of (**2**).‡ The initial rate constants for reactions with (**1**) and (**2**) are quite similar in magnitude in ethanol (10·9% v/v)–dioxan (1·0% v/v)–water (pH 8·12 and μ 0·10 with KCl) at 40 °C: 337 l mol^{-1} s^{-1} for (**1**) and 357 l mol^{-1} s^{-1} for (**2**). A small amount of Cu(NO$_3$)$_2$ drastically reduces the (**2**)-catalysed reaction rate (Figure), suggesting that Cu^{2+} undergoes co-ordination with the imidazole group and hence its nucleophilicity is nearly completely masked. The (**2**)-Cu^{II} complex formed is presumably highly stable since the presence of Cu^{2+} only in twice the amount of (**2**) is sufficient to cause rate-levelling (Figure). The (**1**)-catalysed reaction shows a similar kinetic feature upon addition of Cu^{2+} ion. The rate constant decreases as [Cu^{2+}] increases until it reaches *ca.* $1{\cdot}0 \times 10^{-5}$ mol l^{-1} and further increase in [Cu^{2+}] has no effect on the rate. The Cu^{2+}-saturated rate constant for catalysis by (**1**) is, however, significantly larger (*ca.* 15-fold) than that for catalysis by (**2**). The result strongly suggests that the imidazole group of (**1**) is also co-ordinated to Cu^{2+} and the nucleophilic attack of the oxime group is responsible for the ready deacylation of PNPP; acylation of the oxime group was confirmed by product analysis.§

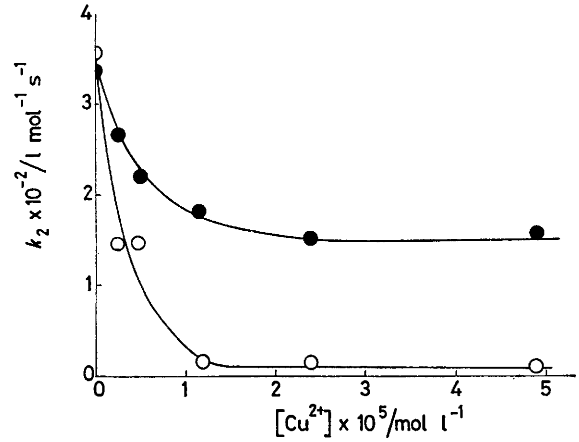


FIGURE. Kinetic effects of Cu^{2+} ion in the deacylation of *p*-nitrophenyl hexadecanoate ($1{\cdot}0 \times 10^{-5}$ mol l^{-1}) as catalysed by (**1**) (●, $5{\cdot}0 \times 10^{-6}$ mol l^{-1}) and (**2**) (○, $5{\cdot}0 \times 10^{-6}$ mol l^{-1}) in ethanol (10·9% v/v)–dioxan (1·0%v/v)–water; pH 8·12 and μ 0·10 (KCl) at 40·0 °C: $k_2 = v_i/([\mathrm{C}][\mathrm{PNPP}])$, where v_i stands for observed initial rate and C for either (**1**) or (**2**).

As for the (**1**)-Cu^{II} complex, both Cu^{2+} ion and the oxime group may be placed in the proximity of each other in accordance with a CPK molecular model, so that some interaction between these would be expected. However, ionization of the oxime group *via* such interaction can be

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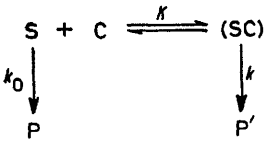
† A hygroscopic material, prepared by oximation of (**2**) with hydroxylamine and purified by repeated high pressure liquid chromatography and gel filtration chromatography; it gave satisfactory analytical and molecular weight data.

‡ The deacylation of PNPP with (**1**) ceases completely upon acylation of the imidazole and oxime groups of (**1**). The rate of acylation of the oxime group is *ca.* 10-fold smaller than that of the imidazole (ref. 1 and the ref. in footnote §).

§ The reaction of (**1**) (18 mg) with PNPP (26 mg) in the presence of Cu(NO$_3$)$_2$ [a 10-fold excess amount over that of (**1**)] was carried out under essentially the same conditions as in the kinetic runs and the resulting cyclophane was purified by gel filtration chromatography: yield 10 mg; ν 1750 cm^{-1} (C=O), indicative of an acylated oxime group (*cf.* Y. Murakami, Y. Aoyama, and K. Dobashi, *J.C.S. Perkin II*, 1977, 32).

ruled out since an ionized [20]paracyclophane oxime loses its nucleophilicity completely upon formation of a stable complex with Cu^{2+}.[3] Consequently, the (**1**)-Cu^{II} complex as its catalytically active form involves the imidazole group co-ordinated to Cu^{2+} and the un-ionized oxime. The reactivity of the oxime group in (**1**)-Cu^{II} is surprisingly high toward PNPP for an un-ionized form and may be assisted by Cu^{2+}. To make this point clear, the pseudo-first-order rate constants (k_{obs}) were determined for deacylation of PNPP ($1{\cdot}0 \times 10^{-6}$ mol l^{-1}) in the presence of various amounts of (**1**)-Cu^{II} at pH 8·12 together with the corresponding rate constants in the presence of the ionized [20]paracyclophane oxime (**3**) measured at pH 11·61.¶ Both systems exhibited typical saturation-type kinetics, consistent with a mechanism which involves pre-equilibrium complexation of cyclophane with substrate (binding constant, K), followed by pseudo-intramolecular acyl transfer (rate constant, k) from the bound substrate to the un-ionized oxime group of (**1**)-Cu^{II} or to the ionized oxime group of (**3**)[4] (Scheme): for (**1**)-Cu^{II} system, $K = 0{\cdot}91 \times 10^5$ l mol^{-1} and $k = 8{\cdot}7 \times 10^{-3}$ s^{-1}; for (**3**), $K = 2{\cdot}1 \times 10^5$ l mol^{-1} and $k = 3{\cdot}8 \times 10^{-3}$ s^{-1}. The Cu^{2+}-assisted nucleophilic reaction of the un-ionized oxime in (**1**)-Cu^{II} with bound PNPP proceeds with a rate constant comparable to or even larger than that for the ionized oxime group in (**3**). The catalytic effect of Cu^{2+} ion cannot be explained in terms of the simple charge effect, since such an unusual nucleophilic reactivity of the un-ionized oxime was not observed for a closely related [20]paracyclophane oxime (**4**) bearing a quaternary ammonium group although (**4**) is a powerful nucleophilic–electrostatic bifunctional catalyst in alkaline media in which the oxime proton is dissociated.[4] A similar [20]paracyclophane oxime (**5**) bearing a protonated imidazole group also failed to exhibit such unusual reactivity.



SCHEME. C, (**1**)-Cu^{II} or (**3**); S, substrate; k_0, rate of spontaneous hydrolysis of PNPP; P, hydrolysis products; P′, acylated cyclophane and phenol.

In conclusion, the present (**1**)-Cu^{II} complex demonstrates the following novel catalytic feature in the light of a suggested mechanism for CPA. (i) A single cyclophane skeleton, which bears both the nucleophile and bound metal, may possess efficient deacylation ability toward a hydrophobic carboxylic ester. (ii) The substrate ester is brought into the proximity of the catalytic centre by the non-covalent hydrophobic interaction, Cu^{2+} playing no significant role in the substrate-binding process since (**1**)-Cu^{II} and (**3**) have comparable binding constants with PNPP. (iii) Reactivity of the poor nucleophile (the un-ionized oxime group) is enhanced most plausibly by marked activation of the substrate carbonyl group through co-ordination with Cu^{2+} ion in the transition state as shown in (**6**).

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¶ Correlations of k_{obs} with concentrations of (**1**)-Cu^{II} and (**3**) were obtained in ethanol (10·9% v/v)–dioxan (1·0% v/v)–water at μ 0·10 (KCl) and 40·0 °C. Maximum concentrations of the catalysts: (**1**), $4{\cdot}6 \times 10^{-6}$ mol l^{-1} ([Cu^{2+}] was maintained constant at $5{\cdot}0 \times 10^{-5}$ mol l^{-1}); (**3**), $4{\cdot}4 \times 10^{-6}$ mol l^{-1}.

[1] Y. Murakami, Y. Aoyama, M. Kida, and A. Nakano, *Bull. Chem. Soc. Japan*, 1977, **50**, 3365.

[2] E. T. Kaiser, *Accounts Chem. Res.*, 1972, **5**, 219; M. W. Makinen, K. Yamamura, and E. T. Kaiser, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 3882.

[3] Y. Murakami, J. Sunamoto, and K. Kano, *Bull. Chem. Soc. Japan*, 1974, **47**, 1238.

[4] Y. Murakami, Y. Aoyama, and K. Dobashi, *J.C.S. Perkin II*, 1977, 24.