# Catalytic Hydrolysis of Amides at Neutral pH

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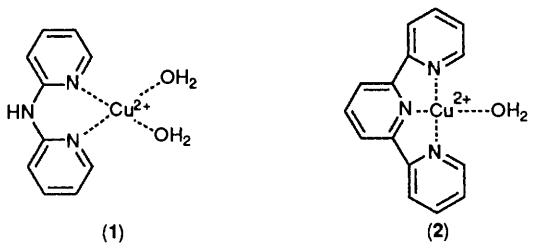
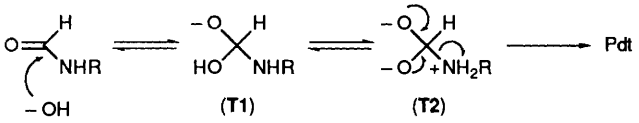
A *cis*-diaqua copper(II) complex, (**1**), has been shown to be active in catalysing the hydrolysis of formamide, *N*-methylformamide, and *N,N*-dimethylformamide in neutral water at 100 °C; monoaqua copper(II) complexes are not active in catalysing the hydrolysis of the amides under the same conditions.

Over the years there has been a great deal of interest in developing artificial enzymes that hydrolyse amides. Owing mainly to the stability of amides and the difficulty associated with monitoring the hydrolysis reaction, most of the artificial enzymes have been tested for hydrolysing *p*-nitrophenyl esters rather than for hydrolysing amides.[1] There is a real need to test the activity of artificial enzymes directly on amides[2,3] since the structural requirements of a catalyst for hydrolysing a *p*-nitrophenyl ester are not the same as that for hydrolysing an amide.[4] Recently, Kahne and Still[2] developed a sensitive assay for detecting trace hydrolysis of unactivated amides. Lerner and Iverson[3] developed a metallocatalytic antibody that hydrolyses amides. In this communication we report on the catalysed hydrolysis of formamides by (**1**) at neutral pH.

The catalysed hydrolysis of formamide, *N*-methylformamide and *N,N*-dimethylformamide by (**1**) was monitored by



(1) (2)



Scheme 1

(CuT2) (CuAc)

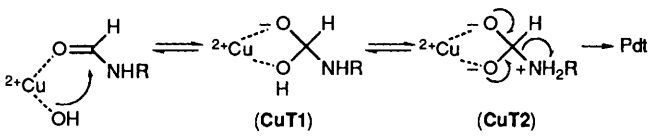
$^1$H NMR spectroscopy. In a typical kinetic experiment, dimethylformamide (10—30 mM) and (**1**) (5—10 mM) in $D_2O$, pD 8.0, were heated at 100 °C. With the progress of the hydrolysis reaction, a decrease in the $^1$H NMR signal due to *N,N*-dimethylformamide [δ 7.92 and δ 2.93, (DSS, 3-(trimethylsilyl)-1-propanesulphonic acid)] is accompanied by an increase in the dimethylamine peak (δ 2.71).† The solution acidity did not change by more than 0.2 pD units throughout the course of the reaction. The rate constants for (**1**) catalysed hydrolysis of formamide, *N*-methylformamide and *N,N*-dimethylformamide are $1.9 \times 10^{-3}$, $6.6 \times 10^{-4}$, and $1.3 \times 10^{-4}$ dm$^3$ mol$^{-1}$ s$^{-1}$ respectively. In sharp contrast to the above results, (**2**) catalysed hydrolysis of the amides could not be detected. The upper limit for (**2**) catalysed hydrolysis of formamide is $1 \times 10^{-5}$ dm$^3$ mol$^{-1}$ s$^{-1}$.

Why should (**1**) be over two orders of magnitude more reactive than (**2**) in hydrolysing the amides? It is unlikely that this difference in the reactivity is due to any steric effect since (**2**) is slightly *more* reactive than (**1**) in hydrolysing *p*-nitrophenyl acetate. Furthermore, it is unlikely that the difference in the reactivity is due to any electronic effect since the difference in the acidity of the water molecules co-ordinated to the metal ion in (**1**) ($pK_a$ = 7.2) and (**2**) ($pK_a$ = 8.0), is less than an order of magnitude. Hydroxide catalysed hydrolysis of amides with poor leaving groups involves addition of the hydroxide to the amide forming the tetrahedral intermediate (**T1**) followed by proton switch (**T2**) and the expulsion of the amine leaving group (Scheme 1).[5]

One way to accelerate the rate of this reaction would be to stabilize (**T2**) thereby increasing its steady-state concentration; (**1**) could stabilize (**T2**) by chelation (**CuT2**). It has been shown by *X*-ray crystallography that (**1**) forms a four-membered ring complex with acetate (**CuAc**).[6]

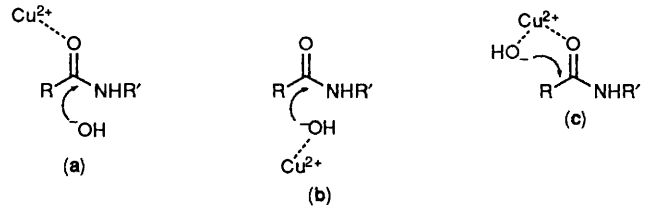
The simplest mechanism that could account for the difference in the reactivity between (**1**) and (**2**) is shown in Scheme 2. Except for the involvement of the metal, the mechanism shown in Scheme 1 is essentially the same as that shown in Scheme 1.

† Formate signal is quenched owing to interaction of formate with the paramagnetic copper complex.

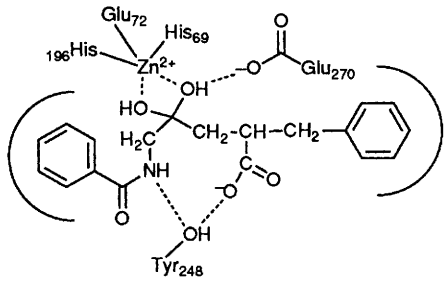


(CuT1) (CuT2)

Scheme 2



(a) (b) (c)



(3)

Kroll's[7] discovery in 1952 of $Cu^{2+}$ catalysed hydrolysis of amino acid esters has triggered an intensive research effort into finding the mechanistic role of the metal ion in this and other hydrolysis reactions[8—10] including those catalysed by hydrolytic metalloenzymes.[11] Two kinetically indistinguishable mechanisms emerged as the two most likely mechanisms for metal-ion promoted hydrolysis of esters and amides. The first is the Lewis acid mechanism involving direct substrate activation (**a**). The second is the metal–hydroxide mechanism involving water activation (**b**). Research teams led by Buckingham,[8] Groves,[9] and Breslow[10] studied enzyme model compounds to show the viability of mechanisms (**a**) and (**b**). Our own interest in determining the structural requirements of a simple metal complex for hydrolysing carboxylate esters,[12] nitriles,[13] and phosphate esters[14] led us to investigate the efficiency of a third mechanism for hydrolysing amides. In this mechanism (**c**) the Lewis acid and the metal–hydroxide mechanisms are combined. If mechanism (**a**) or (**b**) is more efficient than mechanism (**c**) then (**1**) and (**2**) should have comparable reactivities for hydrolysing amides. Although mechanism (**c**) had been considered on paper,[15,16] appropriate model studies supporting the mechanism for amide hydrolysis had not appeared previously.

Although there are similarities between the mechanisms of amide and ester hydrolysis, there are also clear differences.[15] In general, amides are tens of thousands of times less reactive than esters. The rate constants for the hydroxide catalysed hydrolysis of methyl acetate,[17] acetamide[18] and *N,N*-dimethylformamide[19] measured at 25 °C are $1.5 \times 10^{-1}$, $7.4 \times 10^{-5}$, and $1.8 \times 10^{-4}$ dm$^3$ mol$^{-1}$ s$^{-1}$ respectively. The structural requirements of a catalyst for hydrolysing various esters and amides may not necessarily be the same.[4,20,21]

It is impossible to prove enzyme mechanisms based on simple model studies. Nevertheless, detailed mechanistic studies on simple enzyme models can give valuable insights into how enzymes work. Interestingly, the mechanism for (**1**) catalysed hydrolysis of formamides appears to parallel the mechanism for carboxypeptidase A catalysed hydrolysis of

peptides. Christianson and Lipscomb[11] determined the *X*-ray structure (**3**) of a ketone bound to carboxypeptidase A. Surprisingly, the ketone is in its hydrated form with both oxygens of the gem-diol bound to the active site zinc of carboxypeptidase A. The catalysed hydrolysis of formamides by (**1**) and the carboxypeptidase A catalysed hydrolysis of peptides both involve the formation of a four-membered ring bidentate metal complex [copper complex in Scheme 2 and zinc complex in (**3**)]. The crystal structure (**3**) had been used to support mechanism (**b**),[12] but it fits mechanism (**c**) even better. It appears that in some distant past, nature had already chosen the double activation mechanism (**c**).

In conclusion, simple metal complex catalysed hydrolysis of amides in neutral water has been detected. There has been much controversy as to whether the metal ion catalysed hydrolysis of amides would occur by mechanism (**a**) or by mechanism (**b**). This distinction may not be critical since we find that mechanism (**c**) is more efficient than either mechanism (**a**) or (**b**). In terms of catalytic efficiency, (**1**) pales when compared with carboxypeptidase A. Nevertheless, there are interesting similarities between the two catalysts.

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