Pure & Appl. Chem., Vol. 60, No. 4, pp. 489–493, 1988.
Printed in Great Britain.

Kinetics and mechanism of the hydrolysis of functional groups in the side-chain of macrocyclic Cu^{2+}-complexes

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Abstract- A series of functionalized tetraazamacrocycles **2-8** carrying in their pendant side-chain a group which can be hydrolyzed, have been synthesised and their Cu(II) complexes prepared.
The structure analysis of the Cu(II) complex with **5** shows a pentacoordinate Cu(II) surrounded by the four nitrogens of the macrocycle and the carbonyl oxygen of the ester group in a distorted square pyramidal geometry.
Whereas the Cu(II) complexes with the amide derivatives **7** and **8** do not react, the nitrile, the esters of the carbonic acids and of the phosphonate are hydrolyzed in alkaline solution. The kinetics of these reactions were measured and the rate constants determined. The pH-profiles log k/pH are linear with pH for the esters **3-5**, whereas those of the nitrile **2** and the phosphonate ester **6** exhibit a plateau at high pH. This allows to state that the hydrolysis of these last two compounds proceeds through an internal OH^- attack.
For the mechanism of the carbonic acid ester hydrolysis several possibilities are discussed, but no definitive choice is yet possible.

INTRODUCTION

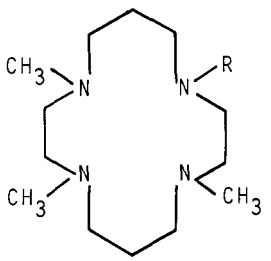
In several esterases and peptidases metal ions play an important role at the active site (ref.1). In general they are essential for activity and it is thought that the metal ion acts as a Lewis-acid and polarizes the bonds of the reactive group so that a nucleophilic attack is facilitated and thus hydrolysis takes place. However, it has also been shown that metal ions can act as organizers, around which the reactands are oriented sterically in such a way that the desired reaction can proceed.

In model reactions both types of mechanisms have also been observed (ref.2). So the hydrolysis of amino acid esters in the presence of Cu(II) is one of the early examples and has extensively been studied (ref.3). For a detailed discussion of the mechanistic pathways, however, the investigation of hydrolytic reactions in the coordination sphere of Co(III) has given the deepest insight (ref.4), since with a kinetically inert metal ion an easier and more direct correlation between structure and reactivity can be made (ref.5). In these examples it has been clearly shown that there are two main possibilities: either a free OH^- attacks the coordinated substrate, which through coordination to the metal ion has been activated, or a coordinated OH^- reacts with the substrate, which is kept in the vicinity of the metal centre. The distinction between external and internal OH^- attack is much more difficult for labile metal ions, since a rapid equilibrium could produce a small amount of the reactive species, the structure of which generally is not easy to determine. To avoid this we have started to use mono-functionalized macrocycles to keep the metal ion in a fixed geometry and at the same time to bring the reactive group close to the metal ion.

We have previously shown that a functional group at the end of the side chain of a tetraazamacrocycle, especially when it has donor properties, can bind to the metal ion when the lenght of the chain is designed in such a way that a five or six-membered chelate ring can be formed (ref.6) and there is no steric interaction which would interfere (ref.7). Such systems seem therefore ideal for the study of metal ion promoted reactions such as hydrolyses. A first example for this type of reactions was the rapid hydrolysis of the

nitrile group in the Cu(II) complex of 2 (ref.8). In this case, although Cu(II) is a labile metal ion, it was clearly demonstrated that the nitrile group is hydrolyzed through the internal attack of an axially coordinated OH^-. It was therefore interesting to study this system with other functional groups, which also can be hydrolyzed and to see whether this same mechanism is also operating. We have prepared for this purpose a series of macrocyclic ligands **3-8** and studied the hydrolysis in their Cu(II) complexes (ref.9).

These compounds have been obtained starting from **1** by alkylation of the secondary nitrogen with the corresponding halogeno derivatives of the esters or amides or by addition of the corresponding acrylo derivatives. In many cases the purification and isolation of the products was only possible after preparing the macrocyclic Cu(II) complexs, which we needed anyway for the kinetical study.



1 R = H
2 R = CH_2-CN
3 R = CH_2-$COOCH_3$
4 R = CH_2-$COOC_2H_5$
5 R = CH_2-CH_2-$COOC_2H_5$
6 R = CH_2-CH_2-$PO(OC_2H_5)_2$
7 R = CH_2-$CONH_2$
8 R = CH_2-$CON(CH_3)_2$

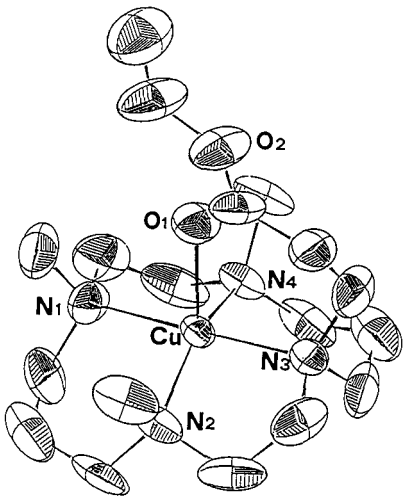


Figure 1
Structure of the Cu(II) complex with **5**

STRUCTURE OF THE Cu^{2+}-COMPLEX WITH 5

From the X-ray structure analysis of the Cu(II) complex with the macrocycle **5** one finds a pentacoordinate Cu(II) surrounded by the four nitrogens of the macrocycle and the carbonyl oxygen of the ester group of the side chain (Figure 1). The Cu-N-bonds are normal (2.07-2.09 Å), whereas that to the carbonyl oxygen is somewhat longer (2.22 Å). The pentacoordination here observed lies between a square pyramidal and a trigonal bipyramidal geometry. The two angles N(1)-Cu-N(3) with 178.6° and N(2)-Cu-N(4) with 155.6° are distinctly different from 180° and 120° expected for a regular trigonal bipyramid and from being all equal as in a square pyramidal environment.

PRODUCTS OF THE HYDROLYSIS REACTION

Whereas the products of the carbonic acid ester hydrolysis are trivial, those of the phosphonate ester and of the nitrile need a short discussion. So in the case of the phosphonate ester complex two consecutive steps (1)

$$Cu(TMC\text{-}PO(OR)_2)^{2+} \longrightarrow Cu(TMC\text{-}PO_2(OR)^{+} \longrightarrow Cu(TMC\text{-}PO_3) \qquad (1)$$

are expected, in which one or both ester groups have been hydrolyzed. The identification of the product produced under the experimental conditions of the kinetical measurements was possible through VIS- and NMR-spectroscopy. During the hydrolysis one observes in a first step a shift of the absorption maximum from 690 nm to 745 nm . A second extremly slow reaction is found at high temperature accompained by a small change in the absorption spectrum. The NMR-spectrum of the product after the first step, having demetalled the complex with KCN, still shows the typical triplet of the methyl group and the quartet of the methylene group of the phosphonate ester. Thus the results from the VIS- and NMR-spectroscopy indicate that the product of the alkaline hydrolysis here studied is the phosphonate mono-ethyl ester.

Similarly there are two possible products in the nitrile hydrolysis: the amide or the acid (2). In order to identify the compound which is formed

$$Cu(TMC\text{-}CN)^{2+} \longrightarrow Cu(TMC\text{-}CONH_2)^{2+} \longrightarrow Cu(TMC\text{-}COO)^{+} \qquad (2)$$

under the experimental conditions of the kinetics, the Cu(II) complex with **2** was hydrolysed in water at 60° and the product isolated. Elemental analysis and IR spectrum indicate the presence of an amide group. In addition the Cu(II) complex was treated with an excess of KCN to destroy the complex and from the alkaline solution the free ligand **7** was extracted with $CHCl_3$ and identified by elemental analysis, IR- and NMR-spectra.

The resulting Cu(II) amide complex is stable even at pH 12 against further hydrolysis, in analogy to the Cu(II)complex with **8**, which does not hydrolyze at all.

KINETICS AND MECHANISM OF THE Cu^{2+}-PROMOTED HYDROLYSIS

Whereas the amide derivatives **7** and **8** do not hydrolyze at the experimental conditions here used, all the other derivatives do show a more or less rapid hydrolytic cleavage. The kinetics were studied either by pH-stat technique (carbonic esters at low pH) or by stopped-flow spectrophotometry (carbonic esters at high pH, phosphonate ester and nitrile). The first method is based on the proton release during the hydrolysis, whereas the second method works, since the spectra of the educts and products are distinctly different, because of the axial coordination of the different pendant groups. The kinetics were followed over the largest possible pH range in order to obtain the maximum of information. The three Figures 2 - 4 show the pH profiles obtained for the carbonic acid esters, the phosphonate and the nitrile, respectively.

A first striking point is the marked difference between the profiles. Whereas all carbonic acid esters follow over the whole pH range a linear dependence on the pH according to eq. 3, the rates of the phosphonate ester and nitrile

$$v = k_1 \cdot [CuTMC\text{-}COOR][OH^-] \qquad (3)$$

hydrolysis have a more complicated pH-dependence. At low pH a linear part can be observed, but at high pH the rate levels off giving a plateau in the pH

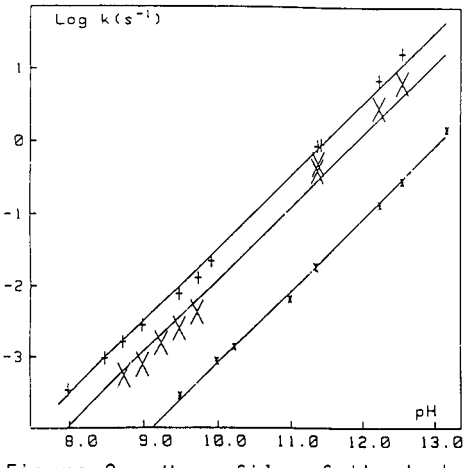


Figure 2. pH-profile of the hydrolysis rate constant for the Cu(II) complexes with **3** (+), with **4** (x) and with **5** (✱)

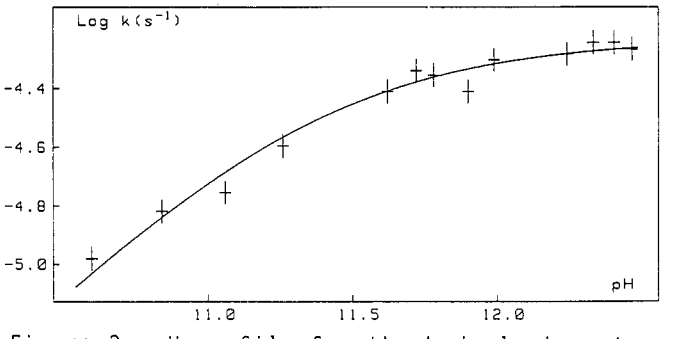


Figure 3. pH-profile for the hydrolysis rate constant of the Cu(II) complex with **6**

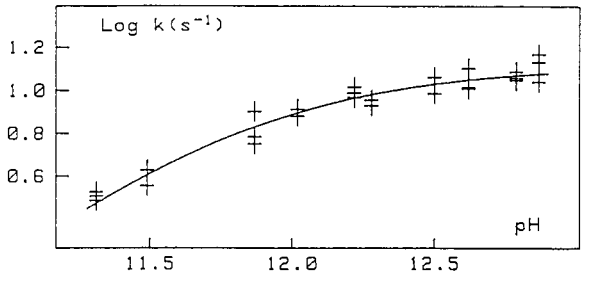


Figure 4. pH-profile for the hydrolysis rate constant of the Cu(II) complex with **2**

profile. This can be explained assuming a rapid equilibrium to a hydroxo species (4) followed by the effective hydrolysis step (5). With this scheme one obtains eq. (6), assuming that the pre-equilibrium (4) is rapid in

$$Cu(TMC\text{-}X)^{2+} + OH^- \rightleftharpoons Cu(TMC\text{-}X)OH^+ \; ; \; K_{OH} \qquad (4)$$

$$Cu(TMC\text{-}X)OH^+ \longrightarrow \text{product} \; ; \; k_2 \qquad (5)$$

comparison with (5). The curves in the Figures 3 and 4 have been calculated using eq.(6).

$$v = \frac{k_2 \cdot K_{OH} \cdot [CuTMC\text{-}X] \cdot [OH^-]}{1 + K_{OH} \cdot [OH^-]} \qquad (6)$$

This type of pH-dependence as well as the observation that SCN^- is an inhibitor of the nitrile hydrolysis allow to propose a mechanism in which the coordinated OH^- attacks the nitrile or the phosphonate ester by an intramolecular process. Thus in these reactions the role of the metal ion is to bring together the two reactands, to organize the transition state and thus to promote the hydrolysis. So the transition state in the nitrile hydrolysis (Figure 5) is probably stabilized by the formation of a five-membered chelate ring in which the amide is generated.

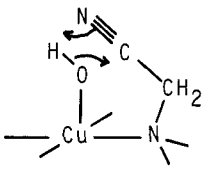


Figure 5. Transition state of the nitrile hydrolysis

Table 1. Bimolecular rate constants for the hydrolysis of the ester group in the Cu(II) complexes and in free amino acid esters at 30°

Compound	$k_1 (M^{-1}s^{-1})$	enhancement
$Cu(\mathbf{3})^{2+}$	231	137
$H_2N\text{-}CH_2\text{-}COOCH_3$	1.69[a]	
$Cu(\mathbf{4})^{2+}$	84	109
$H_2N\text{-}CH_2\text{-}COOC_2H_5$	0.82[b]	
$Cu(\mathbf{5})^{2+}$	5.9	87
$H_2N\text{-}CH_2\text{-}CH_2\text{-}COOC_2H_5$	0.068[c]	

a) From ref.10; b) From ref.11; c) From ref.12. All values extrapolated to 30°

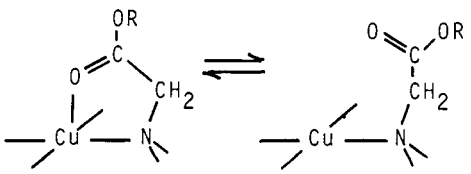


Figure 6. Equilibrium between the open and closed form of the ester

The question whether the carbonic acid esters also react in the same way is not easy to answer. On one side we have the X-ray structure analysis of the solid and the spectral measurements in solution, which indicate that the ester group is coordinated to the metal ion through its carbonyl oxygen, and on the other side the linear pH dependence up to pH 12 - 13. Both these observations could be used to support the external OH^--attack onto the coordinated ester.

A comparison of the bimolecular rate constants k_1 with those obtained for the hydrolysis of amino acid esters gives a rate enhancement of 90 - 140, which although in the right order, because of the polarization of the ester group through the metal ion, is not exceptional.

However, two other possibilities also must be considered. For one there could be a rapid equilibrium between the coordinated and non-coordinated form of the ester (Figure 6) and it could be that the non-coordinated ester group is the reactive one. Nothing quantitative is known about this equilibrium except that it is to a large degree displaced to the left hand side and that

only a small fraction, probably less than 1%, is in the open form. This would mean that the open form would have a reactivity which is at least 100 times higher than the measured one, which in turn would give a rate enhancement of at least 10000 when compared to the free amino acid esters. This is not very convincing and thus we reject this hypothesis.

A further possibility is that the same mechanism as for the nitrile or the phosphonate ester hydrolysis is operating, but that, because of the coordination of the carbonyl oxygen to the axial position and its competition with the OH^-, the hydroxylation equilibrium (4) is displaced to higher pH, so that the plateau in the pH-profile cannot be observed experimentally. The hydrolysis would then proceed by the internal OH^- attack, but there would be only a small amount of the reactive hydroxylated species even at the highest pH studied. Up to now there is no chemical or logical argument to exclude this possibility.

CONCLUSIONS

The examples here studied show that the hydrolysis of several functional groups, such as nitrile, carbonic acid ester and phosphonate ester, but not amide takes place in the Cu(II) complexes at alkaline pH. In two examples it is clear that the mechanism consists in an internal nucleophilic attack of the coordinated OH^- group onto the non-coordinated functional group. This means that the metal ion acts as a matrix to bring close together the nucleophile and the substrate and organizes the transition state. It is possible that in the case of the carbonic acid esters, which are known to coordinate to the Cu(II) in the axial position, the hydrolysis takes place through an external OH^- attack, but this cannot be proven definitively.

Acknowledgement

This work was supported by the Swiss National Science Foundation and this is gratefully acknowledged.

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