Coordination Chemistry Reviews
182 (1999) 243–261

COORDINATION
CHEMISTRY
REVIEWS

Bioinorganic models for the catalytic cooperation of metal ions and functional groups in nuclease and peptidase enzymes

R. Krämer *

Anorganisch-Chemisches Institut der Universität, Wilhelm-Klemm-Str. 8, D-48149 Münster, Germany

Received 20 May 1998; accepted 16 October 1998

Contents

Abstract

Many nuclease and peptidase enzymes achieve highly efficient catalysis by active site cooperation of metal ions and amino acid side chains (acids, bases or nucleophiles). This review focuses on model complexes for which attachment of functional organic groups to a chelate ligand results in an increased hydrolytic reactivity toward phosphodiesters and amides. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Peptidases; Enzymes; Catalysis

---

* Tel.: +49-251-8333158; fax: +49-251-8338366; e-mail: kramerr@nwz.uni-muenster.de.

PII: S0010-8545(98)00235-5

1. Introduction

Nucleases and peptidases are hydrolase enzymes that cleave the two fundamentally important biopolymers, nucleic acids and (poly)peptides. The biological roles of these enzymes include biopolymer degradation/digestion, nucleic acid and protein modification, repair of DNA and viral defence (restriction enzymes). Whereas endonucleases (endopeptidases, respectively) cleave bonds within the polymer chain, exonucleases/peptidases remove terminal nucleotides or amino acids (Fig. 1).

For many nucleases and for some peptidases, metal ions are essential cofactors and are directly involved in the hydrolysis of the phosphodiester or amide bond, respectively.

A growing number of protein crystal structures has provided solid evidence that in most metallo-hydrolases amino acid side chains are involved in substrate transformation as general acids, bases or nucleophiles. Consequently, the high catalytic efficiency is, in part, the result of a perfectly coordinated catalytic cooperation of the metal ion(s) and one or several functional organic groups. Table 1 gives examples of nuclease and peptidase enzymes for which this functional motif

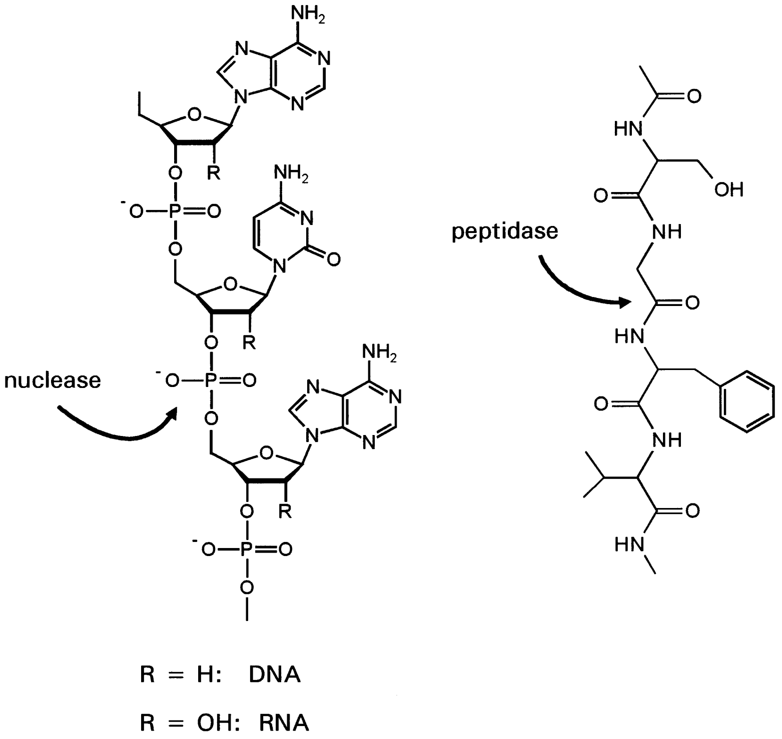


Fig. 1. Structures of nucleic acids and peptides with nuclease and peptidase cleavage sites.

Table 1
Examples of hydrolase enzymes with active site-cooperation of metal ions and functional amino acid side chains

Enzyme	Metal	Functional group (role)	Ref.
Staphylococcal nuclease	Ca^{2+}	2 Arg-guanidinium (acid) Glu-carboxylate (base)	[1–4]
Nuclease S1	Zn^{2+}	Lys-ammonium (acid) Glu-carboxylate (nucleophile)	[5]
Carboxy-peptidase A	Zn^{2+}	Glu-carboxylate (nucleophile or base) Arg-guanidinium (acid)	[6–8]
Leucine aminopeptidase	Zn^{2+}	Lys-ammonium (acid)	[9–12]
Alkaline phosphatase	2 Zn^{2+}	Arg-guanidinium (acid) Ser-alcohol (nucleophile)	[13,14]
Purple acid phosphatase	$Fe^{3+}+Zn^{2+}$	His-imidazolium (acid)	[15,16]
Protein phosphatase 1	$Fe^{3+}+Zn^{2+}$	His-imidazolium (acid)	[17,18]
Urease	2 Ni^{2+}	Lys-ammonium (acid)	[19,20]

was proposed, mostly on the basis of crystallographic evidence. Other hydrolases that cleave phosphate monoesters (phosphatases) and the amide bond of urea (urease) are also included.

The generally accepted mechanisms of phosphodiester and amide hydrolyses by hydroxide are shown in Fig. 2:

At pH 7 and 25°C phosphodiesters and amides are very stable, the half lifes for hydrolysis are estimated 2000 years for amides [21], 100 years for RNA [22] and 200 million years for DNA [23]. The higher reactivity of RNA compared with DNA is due to the presence of the ribose 2′-OH group which may act as an intramolecular nucleophile. One important way to promote the hydrolysis reactions is the compensation of negative charge in the tetrahedral intermediate of amide cleavage or the trigonal-bipyramidal transition state of phosphodiester cleavage.

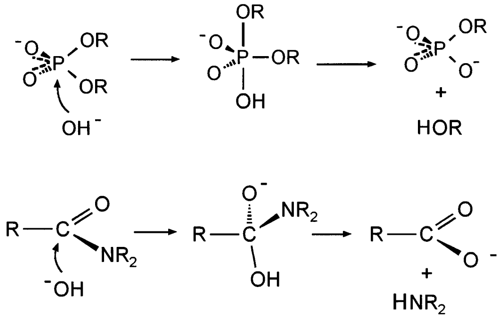


Fig. 2. Mechanism of phosphodiester (top) and amide (bottom) bond cleavage by hydroxide.

Metal ions and functional organic groups can promote hydrolytic cleavage in the following ways:

- Electrostatic activation of the substrate in the ground state or stabilization of the transition state by metal ion coordination, hydrogen bonding or proton transfer.
- Stabilization of leaving group by metal coordination, hydrogen bonding or proton transfer.
- Nucleophilic attack on the substrate by functional group or by metal-coordinated hydroxide, which is generated at neutral pH by Lewis-acid activation of metal coordinated water.
- Generation of the nucleophile via proton abstraction by a basic group

Low molecular weight mimics for nucleases and peptidases are relevant from several perspectives. First of all, it is easier to study mechanistic features on a simple model system than on the enzyme itself. The models may provide valuable information for understanding the chemistry involved in the active site. Second, in view of biochemical applications there is substantial interest in artificial nucleases and peptidases which are smaller, more robust and better available than the enzymes themselves. Combined with recognition agents, e.g. antisense oligonucleotides, artificial nucleases are useful for the nonoxidative, sequence specific cleavage of nucleic acids and might become important tools in future biotechnology [24]. Application of such reagents as drugs for the selective control of gene expression have also been envisaged [25–27]. Artificial peptidases could be useful in elucidating the solution structure of proteins as well as in the sequencing of large proteins.

For the hydrolysis of both phosphodiester and amide bonds under mild conditions, metal ions and metal complexes are the most efficient nonenzymatic reagents currently available. However, they do not reach the catalytic efficiency of enzymes, and higher reactivities are desirable in view of applications. Functionalisation of metal complexes with organic, catalytic auxiliary groups is a strategy to generate more efficient artificial nucleases and peptidases.

This review focuses on model complexes in which covalent attachment of a functional (acidic, basic, nucleophilic) group results in a significant increase of reactivity by direct or indirect interaction with the hydrolyzable group.

Systems in which substrate binding and orientation is achieved by hydrophobic interaction with attached nonpolar groups (e.g. cyclodextrins [28]) are not included in this review. Also, reports on intermolecular synergistic effects of metal ions and acids, bases or nucleophiles are not considered.

2. Metal-functional group cooperation in nucleases and peptidases

2.1. Nucleases

Staphylococcal nuclease is an extracellular endonuclease which cleaves the phosphodiester backbone of DNA and RNA to yield 3′-mononucleotides. It is a digestive enzyme without sequence specificity but with enormous catalytic efficiency. DNA hydrolysis is accelerated 10^{16}-fold. This is probably the highest rate

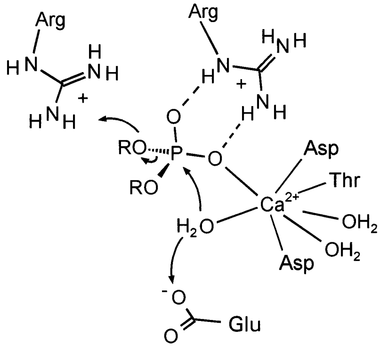


Fig. 3. Reaction mechanism of staphylococcal nuclease.

enhancement reported for enzymatic phosphodiester cleavage. The structure of the active site with a bound nucleotide 3′,5′-diphosphate has been elucidated by X-ray crystallography [1]. Also, detailed mechanistic studies have been performed, e.g. by site directed mutagenesis [2–4]. There is good evidence for the reaction mechanism shown in Fig. 3. The phosphodiester group is activated electrostatically by coordination of the Ca^{2+} ion and hydrogen bonding with one Arg-guanidinium group. The nucleophile which attacks the phosphorus atom might either be a calcium coordinated (as shown in Fig. 3) or a free water molecule which is deprotonated by a Glu-carboxylate group. A second Arg-guanidinium stabilizes the trigonal-bipyramidal transition state.

Nuclease S1 from *Aspergillus oryzae* is probably a trinuclear zinc enzyme [29]. Biological function and substrate specificity are similar to staphylococcal nuclease. The mechanism proposed in Fig. 4 is based on biochemical studies alone and is more speculative [5]. It was suggested that a Zn^{2+} ion and a Lys-ammonium group interact with the phosphodiester group and facilitate the attack of a nucleophile, possibly a Glu-carboxylate group. The mixed anhydride is cleaved by a zinc-coordinated hydroxide, yielding a phosphate monoester and regenerating the carboxylate group.

2.2. Peptidases

Carboxypeptidase A (CPA) from bovine pancreas hydrolyses the amide bond of the C-terminal amino acid of peptides, in particular in the presence of large

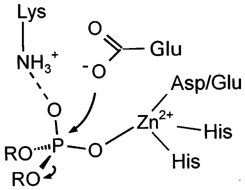


Fig. 4. Reaction mechanism of nuclease S1.

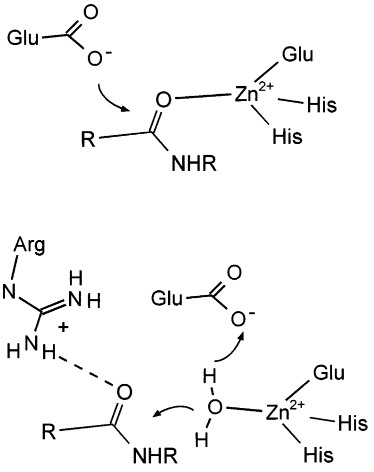


Fig. 5. Reaction mechanisms proposed for carboxypeptidase A, top: carboxylate nucleophile, bottom: Zn–OH nucleophile.

amino-acid side chains such as the benzyl substituent in phenylalanine. The mechanism of CPA has been the centre of controversy in spite of intensive investigation. Crystal structures are available both for the substrate free enzyme and for several enzyme-inhibitor complexes [6]. One mechanistic idea includes ground state activation of the amide by coordination of the carbonyl oxygen to Zn^{2+}, followed by nucleophilic attack of Glu-carboxylate (Fig. 5, top) [7,8]. The anhydride intermediate is hydrolysed by a zinc-coordinated hydroxide.

An alternative proposal (Fig. 5, bottom) favours a Zn–OH nucleophile which directly attacks the amide group [6]. Furthermore, the carbonyl group is activated by hydrogen bonding with Arg-guanidinium. The role of Glu-carboxylate is deprotonation of zinc coordinated water (formation of Zn–OH) and, subsequently, leaving group stabilization by proton transfer to the amine.

Leucine aminopeptidase is an exopeptidase that catalyzes the removal of amino acids from the N-terminus of a peptide. Crystal structures have been reported both for the substrate-free enzyme [10] and for enzyme–inhibitor complexes [11,12]. Based on these structures a reaction mechanism is currently favoured where two Zn^{2+} ions and a Lys-ammonium group are directly involved in amide bond

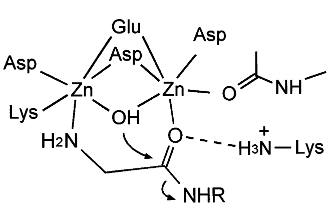


Fig. 6. Reaction mechanism of leucine aminopeptidase.

cleavage (Fig. 6). One of the metal ions acts as a Lewis acid and electrostatically activates the carbonyl group. An additional electrostatic activation is achieved by hydrogen bonding of a Lys-ammonium group with the carbonyl oxygen. A hydroxide that bridges the two zinc ions acts as a nucleophile and attacks the carbonyl group.

3. Model complexes

3.1. Substrates

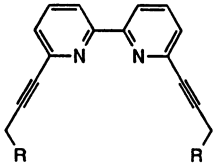
The natural substrates of nuclease enzymes, RNA and in particular DNA, are very resistant to hydrolysis. There are only few examples for a detectable nonenzymatic hydrolysis of linear DNA under mild conditions. Therefore, many model studies use activated *p*-nitrophenyl esters which are more reactive. Often, the trends in reactivity observed with activated substrates can be transferred to their nonactivated counterparts [30]. This is not the case, however, if leaving group stabilization is important since the K_a of a nitrophenol leaving group is about 7 orders of magnitudes smaller than the K_a of an aliphatic alcohol. Spontaneous cleavage of RNA is much faster compared to DNA since the ribose 2′-OH group acts as an intramolecular auxiliary group. 2-Hydroxypropyl-*p*-nitrophenyl phosphate (HPNP), an activated RNA-analog, releases *p*-nitrophenolate by intramolecular nucleophilic attack of the OH-group and formation of a cyclic phosphodiester.

The phosphodiester substrates used for the model studies described herein are shown in Fig. 7.

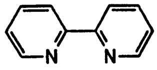
3.2. Nuclease models

3.2.1. Metal–acid cooperation

We have designed a simple model system to mimic the cooperativity of a metal ion and a NH-acidic group in phosphodiester hydrolysis [31–34], as proposed for staphylococcal nuclease and nuclease S1. In $\mathbf{L}^1$ two ammonium groups are linked to a metal binding 2,2′-bipyridine (bpy) unit by two propyne spacers. The choice of the spacing group was of particular importance since an intramolecular chelation of the pendant functional group has to be prevented. On the other hand, steric preorganization of the ammonium groups should facilitate interaction with metal bound phosphate ester. For comparison, the related ligands $\mathbf{L}^2$ and $\mathbf{L}^3$ were prepared.



L^1: $R = NMe_2H^+$
L^2: $R = Et$
L^3: $R = NMe_3^+$



2,2′-bipyridine (bpy)

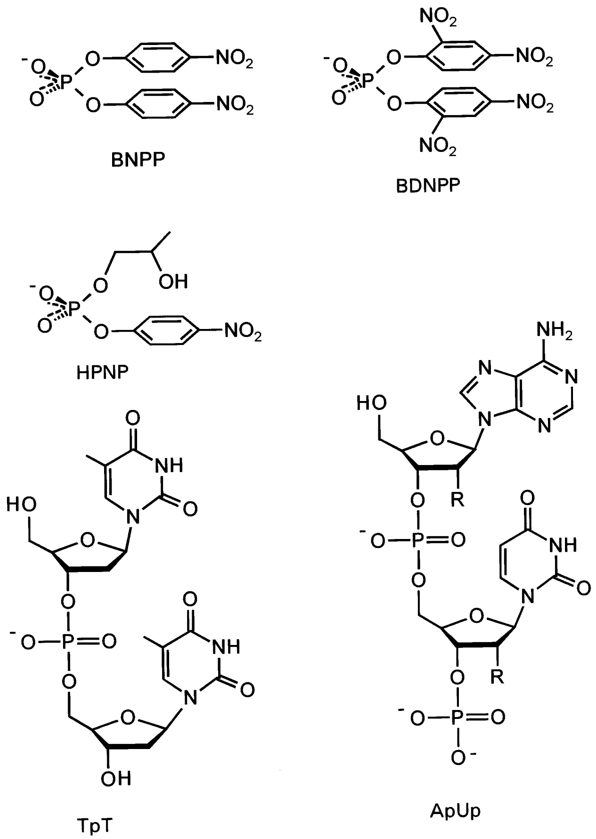


Fig. 7. Phosphodiester substrates used for kinetic studies with model complexes.

In a crystal of $(\mathbf{L}^2)ZnCl_2$ (Fig. 8) the zinc ion is tetrahedrally coordinated [31]. Molecular models indicate that for other normally encountered geometries of Zn (trigonal-bipyramidal, octahedral) a significant steric hindrance of the coordination sphere with the alkyne substituents of **L** is expected. In the absence of ligand field

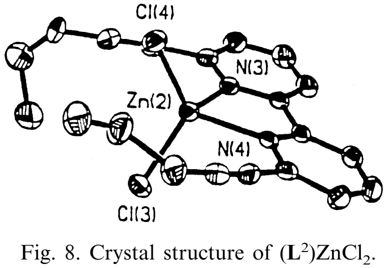


Fig. 8. Crystal structure of $(\mathbf{L}^2)ZnCl_2$.

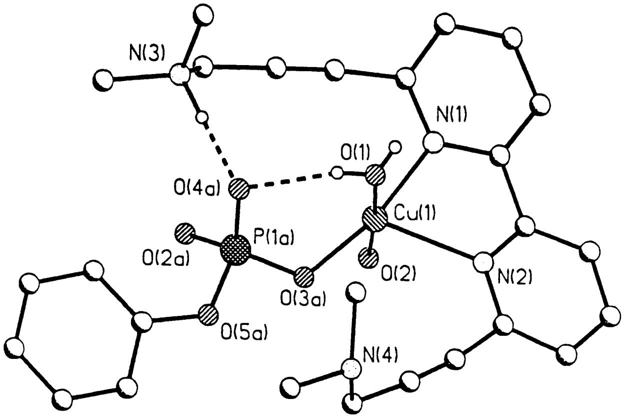


Fig. 9. Crystal structure of mononuclear subunit of the centrosymmetric complex $[Cu_2(\mathbf{L}^1)_2(1,3\text{-}\mu\text{-}PO_3OPh)_2(OH_2)_2](NO_3)_4$.

stabilization those geometries are clearly disfavoured, and the tetrahedral coordination should be retained in solution.

The copper(II) complexes $[Cu(\mathbf{L}^1)(NO_3)(OH_2)_2](NO_3)$ [32], $[Cu_2(\mathbf{L}^1)_2(1,3\text{-}\mu\text{-}PO_3OPh)_2(OH_2)_2](NO_3)_4$ [33] (Fig. 9), and $[Cu_2(\mathbf{L}^2)_2CuCl_4]$ [35] were characterized crystallographically. In all three compounds the metal is square-pyramidally coordinated with an in-plane O_3N (Cl_3N, respectively) donor set and a longer bond to one bpy nitrogen in the apical position. This structure is different from (bpy)Cu complexes (e.g. (bpy)$CuCl_2$) where both Cu–N bonds are short and lie in the equatorial plane. For (**L**)Cu such a geometry is disfavoured by the steric hindrance of coligands with the alkyne substituents of **L**.

The structure with the coordinated monoester phenylphosphate is particular informative in view of the possible interaction of the complex with phosphodiester substrates. Phenylphosphate is coordinated to the metal ion by O(3a) and additionally forms a hydrogen bond with one of the ammonium groups by O(4a). Such a bifunctional interaction would result in an efficient electrostatic activation of the substrate and facilitates nucleophilic attack at phosphorus. Furthermore, the copper coordinated water molecule O(1) is only 3.2 Å apart from the phosphorus atom. Apparently, hydrogen bonding with $-NMe_2H^+$ brings the substrate in a suitable orientation for metal–hydroxide nucleophile which can be provided by deprotonation of coordinated water.

According to EPR spectroscopy the coordination geometry of ($\mathbf{L}^1$)Cu in a frozen reaction solution is similar to that in the solid state. Whereas bpy forms stable 1:1-complexes the attack of a with Zn^{2+} and Cu^{2+} in aqueous solution the complexes of **L** have a surprisingly low stability. To achieve complex formation it was necessary to use organic-aqueous solvent mixtures. The zinc(II) complexes were investigated in acetonitrile–water 19:1 (v/v) and the copper complexes in ethanol–water 19:1 (v/v).

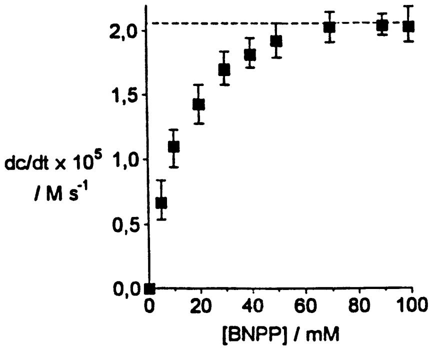


Fig. 10. Saturation kinetics for the hydrolysis of BNPP by ($\mathbf{L}^1$)Cu (5 mM). Reaction conditions: ethanol–water 19:1, 20°C, pH 6.6.

Cleavage of phosphodiesters was studied using the activated DNA-analog bis(*p*-nitrophenyl)phosphate (BNPP) (Fig. 7). For short reaction times ($>$ 5% conversion of BNPP) the monoester *p*-nitrophenyl phosphate is the only reaction product and no PO_4^{3-} is formed according to ^{31}P NMR. ($\mathbf{L}^1$)Zn is at least five times more reactive than ($\mathbf{L}^2$)Zn and (bpy)Zn.

For ($\mathbf{L}^1$)Cu a plot of rate versus substrate concentration (Fig. 10) gave good Michaelis–Menten behaviour with $k_{cat} = 4.4(\pm 0.4) \times 10^{-3}$ s^{-1} and $K_M = 0.009$ M at pH 6.6 and 20°C. BNPP bound to ($\mathbf{L}^1$)Cu hydrolyzes 4×10^7 times faster than free BNPP [$k_{uncat} = 1.1(\pm 0.3) \times 10^{-10}$ s^{-1}] under the same conditions.

For ($\mathbf{L}^3$)Cu and for (bpy)Cu the values of k_{cat} are much lower, and the metal-free ligand $\mathbf{L}^1$ has no effect at all on the hydrolysis rate (Table 2). ($\mathbf{L}^1$)Cu is 1000 times more reactive than ($\mathbf{L}^3$)Cu and 3400 times more reactive than (bpy)Cu at pH 6.6.

For ($\mathbf{L}^1$)Cu the reaction rate increases linearly with pH and reaches a plateau at ca. pH 6.0. Above pH 6.8 precipitates form. A similar pH rate profile is observed for (bpy)Cu. A pH titration of ($\mathbf{L}^1$)Cu in the presence of the less reactive diphenylphosphate indicates abstraction of 0.7 equivalents H$^+$ at pH 6.6

Table 2
Relative reactivities (k_{rel}) of copper complexes toward BNPP[a]

Catalyst	k_{cat} (s^{-1})	k_{rel}
[($\mathbf{L}^1$)Cu]	$4.4(\pm 0.4) \times 10^{-3}$	4×10^7
[($\mathbf{L}^3$)Cu]	$4(\pm 1) \times 10^{-6}$	4×10^4
(bpy)Cu	$1.2(\pm 0.2) \times 10^{-6}$	1.1×10^4
[$\mathbf{L}^1$](NO_3)$_2$	$1.2(\pm 0.3) \times 10^{-10}$	1.1
None	$1.1(\pm 0.3) \times 10^{-10}$	1

[a] k_{cat} represents the first-order rate constant for hydrolysis of BNPP which is coordinated to the catalysts. Reaction conditions: 5 mM catalyst, ethanol–water 19:1, 20°C, pH 6.6.

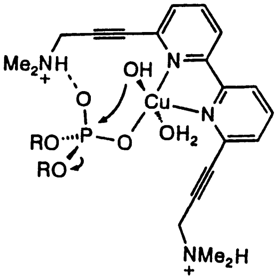


Fig. 11. Plausible reaction mechanism for the hydrolysis of BNPP by (**L**^{1})Cu.

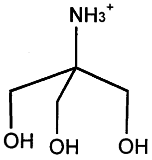
in ethanol water 19:1. Since metal-coordinated water and the ammonium groups might have similar pK_a values it cannot be decided which group releases the proton. The ammonium groups of the free ligand have p$K_a = 7.2$ under the same conditions.

Although catalytic turnover is observed with (**L**^{1})Cu the complex is not an efficient catalyst since the reaction product nitrophenyl phosphate strongly binds to the complex and inhibits the reaction. The addition of one equivalent of nitrophenyl phosphate reduces the reaction rate by $> 50\%$.

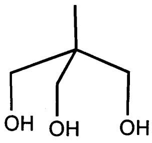
(**L**^{1})Cu is much more reactive than the corresponding zinc complex. Possibly this is a consequence of the different coordination geometries at the metal ion. According to molecular models metal hydroxide attack at the phosphorus atom is very much disfavoured in the tetrahedral zinc complex for steric reasons.

For hydrolysis of BNPP by (**L**^{1})Cu we suggest that the phosphodiester interacts with the complex both by metal coordination and by hydrogen bonding with one ammonium group. This bifunctional interaction results in a double electrostatic activation of the substrate. Additionally, the substrate is brought in proper orientation for nucleophilic attack of copper coordinated hydroxide at the phosphorus atom (Fig. 11).

Recently, we described another system for which the presence of an ammonium-functionality in the ligand leads to increased reactivity toward phosphodiesters. We detected that Zr(IV) salts in weakly acidic aqueous solution at 20°C efficiently cleave the activated phosphodiester BNPP and the DNA dinucleotide thymidyl–thymidine (TpT, Fig. 7) [36]. Among many ligands investigated, only tris(hydroxymethyl)aminomethane (**L**^{4}) formed a complex which had a distinctly higher reactivity than the metal salt alone at pH 3.5. The 1:1 **L**^{4}–Zr complex is about 1.5 times more reactive in the cleavage of both BNPP and TpT. We suggest tridentate coordination of **L**^{4} by three alcohol (alcoholate, respectively) groups [36] whereas the $-NH_2$ group should be protonated at pH 3.5. In contrast, the addition of tris(hydroxymethyl)ethane (**L**^{5}) to the Zr(IV) salt does not result in better reactivity. (**L**^{1})Zr accelerates hydrolysis of the DNA dinucleotide TpT 3×10^9-fold and is among the most reactive artificial DNAs ever reported. Whether the increased reactivity is owing to direct interaction of the ammonium group with the phosphodiester substrate has to be clarified by more detailed investigation of this system.



L⁴

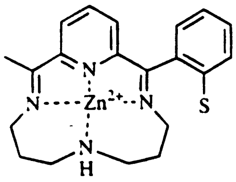


L⁵

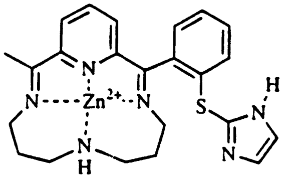
3.2.2. Metal–base cooperation

Several model systems have been described for which a intramolecular metal base cooperation has been demonstrated in the cleavage of the RNA-mimic 2-hydroxypropyl(*p*-nitrophenyl)phosphate (HPNP, Fig. 7) or of RNA-dinucleotides. Although these systems are not directly related to the enzymes listed in Table 1 they show that a basic auxiliary group may lead to a significant increase in cleavage rate, presumably by generating an intramolecular alcoholate nucleophile by proton abstraction from the substrate alcohol group.

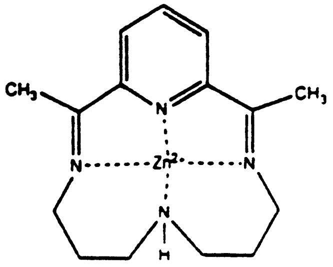
Breslow and coworkers [37] have synthesized macrocyclic zinc(II) complexes with attached thiophenol (**1**) or imidazole (**2**) groups and have compared them with the unfunctionalized analog **3**. The catalytic auxiliary groups are held so that they can not directly bind to the metal.



1



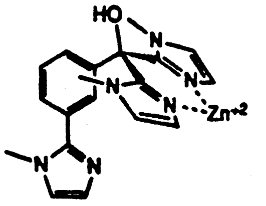
2



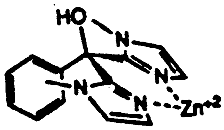
3

In water at pH 7 and 37°C the thiophenol (thiophenolate, respectively) and imidazole group increase the effectiveness for HPNP transesterification by a factor of 9 and 20, respectively. The suggestion that the auxiliary groups provide general base catalysis is based on the pH rate profile.

Another imidazole functionalized Zn complex **4** was reported by Anslyn and coworkers [38]. For the transesterification of the RNA-dinucleotide ApUp (Fig. 7) **4** is superior to its unfunctionalized analog **5** by a factor of 1.5. It was suggested that reaction is supported either by general base (imidazole) or general acid (imidazolium) catalysis.

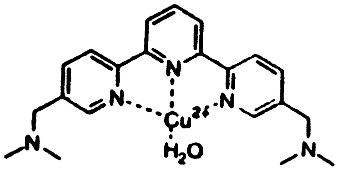


4

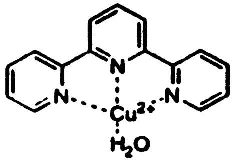


5

Hamilton and coworkers have attached tertiary amino groups to a copper(II) terpyridine complex [39]. In water at pH 7.0 **6** cleaves HPNP seven times faster than [(terpyridine)Cu]$^{2+}$ (**7**). The pH rate profile indicates that the amino groups acts as an auxiliary base, presumably by deprotonation of the hydroxyl group of HPNP.



6

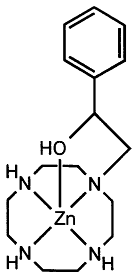


7

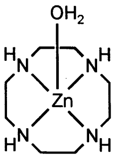
3.2.3. Metal–nucleophile cooperation

Recently several model studies have focused on metal complexes with a coordinated alcoholate nucleophile. There is no direct relation to phosphodiesterase enzymes, but nucleophilic attack of zinc coordinated serine alcoholate to a phosphate monoester is a generally accepted functional motif of alkaline phosphatase, a well investigated dizinc(II) phosphomonoesterase [13,14]. Phosphate is released in a second step by nucleophilic attack of zinc-hydroxide to the serine phosphate intermediate.

Hydrolysis of BNPP by the macrocyclic Zn(II) complex **8** with a benzyl alcohol pendant has been studied in detail by Kimura et al. [40]. The coordinated alcohol group is strongly activated by the Zn^{2+} Lewis acid and has a pK_a value of 7.4. Nucleophilic attack of coordinated Zn–alcoholate to the P-atom of BNPP results in transesterification and formation of an intermediate in which the phosphate is covalently attached to the ligand. BNPP cleavage in water at pH 9.3 and 35°C is 125 times faster than hydrolysis by Zn–OH in complex **9**, indicating that the alcoholate is a more potent nucleophile. Another *p*-nitrophenol is released from the *p*-nitrophenylphosphoryl derivative of **8** by nucleophilic attack of zinc coordinated hydroxide. The final product of the stoichiometric reaction sequence is a phosphomonoester complex.

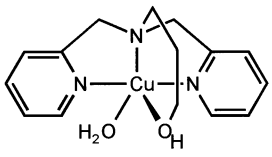


8

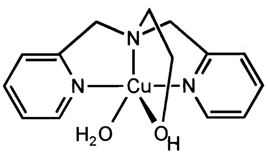


9

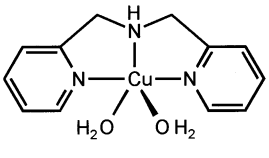
Chin and coworkers have investigated the reactivity of copper complexes **10**, **11** and **12** toward substrate bis(2,4-dinitrophenyl)phosphate (BDNPP) [41]. In water at pH 8.8 and 25°C alkoxide complex **10** is 30 times more reactive than the hydroxo complex **12**. The proposed reaction mechanism is different from that of complex **8** where reaction with phosphodiesters is intermolecular. In contrast, the BDNPP substrate replaces a water molecule in complex **10** and Cu–alkoxide nucleophilically attacks the coordinated phosphodiester. No further reaction is observed for the product of the stoichiometric transesterification reaction in which dinitrophenyl phosphate is covalently attached to the ligand. Interestingly, complex **11** with the hydroxyethyl group is about 100 times less reactive than **10** and cleaves BDNPP predominantly by attack of copper coordinated hydroxide (Fig. 12). This is explained by the different structures of the complexes. apparently, alkoxide attack to the coordinated phosphodiester is facilitated by the larger chelate ring size in hydroxypropyl complex **10.**



10



11



12

Morrow et al. [42] have prepared macrocyclic complexes **13** of lanthanide ions Eu^{3+} and La^{3+} and have tested their reactivity toward BNPP. As for the above mentioned copper complex **10** nucleophilic attack of metal–alkoxide to the coordinated substrate is proposed. Again, the product of transesterification is stable and

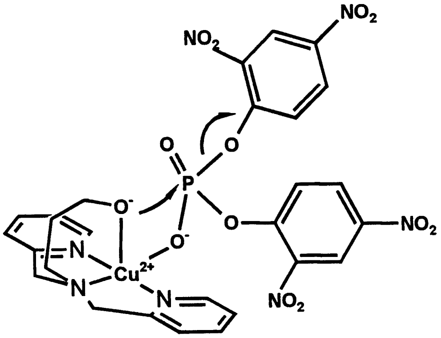
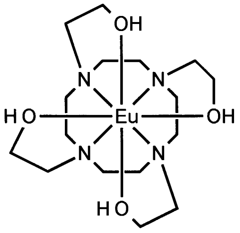


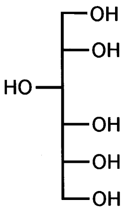
Fig. 12. Proposed mechanism of BDNPP cleavage by **10**.

does not release a second equivalent of nitrophenyl phosphate. The europium complex **13** at 1 mM concentration at pH 7.4 and 25°C gives a rate enhancement of nearly 10^7 over the uncatalyzed hydrolysis reaction of BNPP. Kinetic data for a related Eu(III) macrocyclic complex without pendant alcohol groups are not available since the unfunctionalized tetraaza macrocycle does not form stable Eu(III) complexes in water.

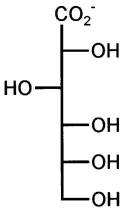


13

Schneider and coworkers have studied the effect of various polyol ligands on lanthanide(III) ion promoted cleavage of BNPP [43] and supercoiled plasmid DNA [44]. The Eu(III) complex of $\mathbf{L}^6$ is 2.5 times more reactive toward BNPP than $EuCl_3$. In the linearization of supercoiled plasmid DNA by La^{3+} ligand, $\mathbf{L}^7$ leads to about a four-fold increase of rate beyond the metal alone at pH 7 and 37°C. It is proposed that the nucleophilic species in these reactions are metal coordinated alkoxides.



$\mathbf{L}^6$



$\mathbf{L}^7$

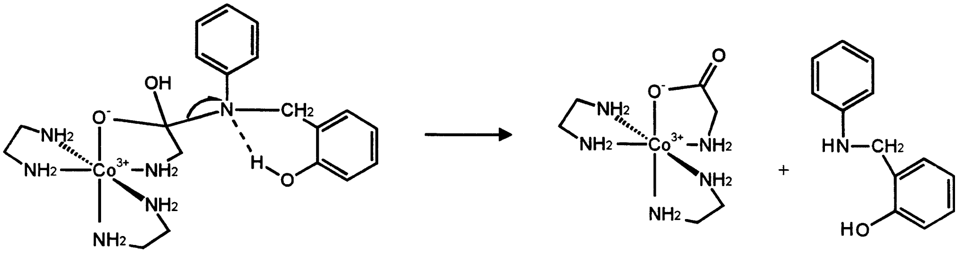
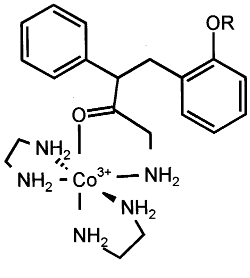


Fig. 13. Mechanism of amide bond cleavage in **14** (nucleophilic attack of external hydroxide)

3.3. Peptidase models

3.3.1. Metal–acid cooperation

To mimic certain mechanistic features of carboxypeptidase A (CPA) Breslow et al. have prepared the cobalt(III) complex **14** with *O*-coordinated amide group and a phenol moiety covalently attached in proximity to the amide [45]. At the time when this model system was designed it was considered that in CPA a tyrosine phenolic hydroxyl group has an important function in the stabilization of the amine leaving group via hydrogen bonding. Later, site directed mutagenesis has revealed that replacement of Tyr by Phe does not result in a decrease of catalytic efficiency. Thus, any mechanisms that utilize the Tyr-OH group are invalid. Nevertheless, **14** represents an interesting example for the cooperation of a metal ion and an acidic functional group in amide hydrolysis. The amide group is strongly activated by coordination of the carbonyl oxygen to the cobalt (III) ion for nucleophilic attack by an external hydroxide ion. Compared with complex **15** where the phenolic OH is replaced by OMe, the functional OH group in **14** enhances the cleavage rate by a factor of 100 between pH 7.5 and 9 in water–DMSO mixtures. Presumably, the phenol–OH facilitates release of the leaving group by protonation of the amine nitrogen (Fig. 13). Breslows group has also prepared a model complex in which the phenolic OH is replaced by carboxylate. Although the carboxylate group is in a proper position for nucleophilic attack to the coordinated amide, there was no intramolecular catalysis by the acetate group.

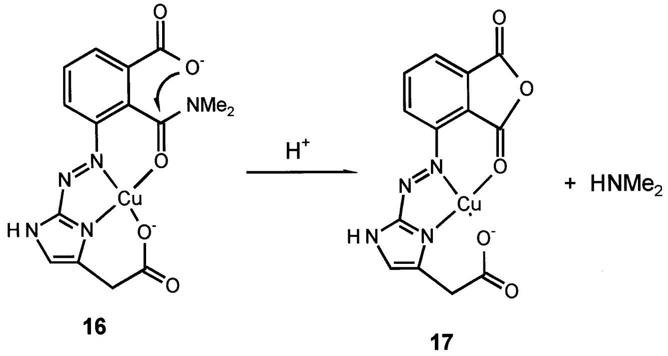


14: R = H

15: R = Me

3.3.2. Metal–nucleophile cooperation

Suh and coworkers [46–49] have described model systems for the cooperation of a divalent metal ion and a carboxylate nucleophile in amide and ester hydrolysis, suggested as a mechanistic feature of carboxypeptidase A. For example, the amide bond in the copper(II) complex **16** has a half life of 10 min at 50°C in a DMSO/water (95/5) medium. This corresponds to a ca. 300-fold rate enhancement compared with the background reaction in the absence of metal ion and carboxylate. The cyclic anhydride intermediate **17** is rapidly hydrolysed. In view of the much greater stability of amide bonds compared with ester bonds it is noteworthy that the hydrolysis rate constants of **16** and of the methyl ester analog of **16** are comparable. This is another feature of carboxypeptidase A reproduced by the model.



In the peptidase models described above the functional organic group is covalently linked to the substrate. Therefore, these systems are not directly relevant to the rational design of metal complexes that catalyse peptide hydrolysis intermolecularly. Nevertheless, it is a valuable observation that both acidic and nucleophilic auxiliary groups can substantially increase the efficiency of metal-promoted amide cleavage.

4. Conclusions

Cooperation of an active site metal ion with acidic, basic or nucleophilic amino acid site chains is a common functional motif in nuclease and peptidase enzymes. In recent years several metal complexes that model this feature have been prepared by attachment of a functional organic group to the metal chelating ligand. For the hydrolysis of phosphodiesters and amides rate increases relative to the unfunctionalized complexes have been achieved by auxiliary acids (ammonium, phenol–OH), bases (imidazole, amine, thiophenolate) or nucleophiles (carboxylate, alkoxide). The model complexes are listed in Table 3.

Table 3
Model complexes considered in this review

Complex	Metal	Auxiliary group (role)	Substrate	Rate increase[a]	Ref.
$(\mathbf{L}^1)$Zn	Zn^{2+}	Ammonium (acid)	BNPP	5	[31]
$(\mathbf{L}^1)$Cu	Cu^{2+}	Ammonium (acid)	BNPP	1000	[32–34]
$(\mathbf{L}^4)$Zr	Zr^{4+}	Ammonium (acid?)	TpT, BNPP	1.5	[36]
1	Zn^{2+}	Thiolate (base)	HPNP	9	[37]
2	Zn^{2+}	Imidazole (base)	HPNP	20	[37]
4	Zn^{2+}	Imidazole (base or acid)	ApUp	1.5	[38]
6	Cu^{2+}	Amine (base)	HPNP	7	[39]
8	Zn^{2+}	Alkoxide (nucleophile)	BNPP	125[b]	[40]
10	Cu^{2+}	Alkoxide (nucleophile)	BDNPP	30[b]	[41]
13	Eu^{3+}	Alkoxide (nucleophile)	BNPP	[c]	[42]
$(\mathbf{L}^6)$Eu	Eu^{3+}	Alkoxide (nucleophile?)	BNPP	2.5	[43]
$(\mathbf{L}^7)$Eu	Eu^{3+}	Alkoxide (nucleophile?)	DNA4	[44]	
14	Co^{3+}	Phenol–OH (acid)	Amide	100	[45]
16	Cu^{2+}	Carboxylate (nucleophile)	Amide	[c]	[46–49]

[a] Increase by auxiliary group relative to the unfunctionalized complex.
[b] Relative to the hydroxo-complex.
[c] No comparative system available.

The rate enhancements ranges from 1.5- to 1000-fold for the ammonium-functionalized complex $(\mathbf{L}^1)$Cu. Reactivity of the model complexes seems to be strongly influenced by the nature of auxiliary group, its steric orientation relative to the metal ion, and the flexibility of the linker that connects the group with the chelate ligand. The design of future model systems of this type should be directed to a better understanding of the factors that produce large cooperative effects.

References

[1] F.A. Cotton, E.E. Hazen Jr., M.J. Legg, Proc. Natl. Acad. Sci. USA 76 (1979) 2555.
[2] D.J. Weber, A.K. Meeker, A.S. Mildvan, Biochemistry 30 (1991) 6103.
[3] E.H. Serpersu, D. Shortle, A.S. Mildvan, Biochemistry 26 (1987) 1289.
[4] C.B. Anfinsen, P. Cuatrecasas, H. Taniuchi, Enzymes 4 (1971) 177.
[5] H. Witzel, W. Berg, O. Creutzenberg, A. Karreh, in: I. Bertini, C. Luchinat, W. Maret, M. Zeppezauer (Eds.), Zinc Enzymes, Birkhäuser, Boston, 1986, pp. 295–306.
[6] D.W. Christianson, W.N. Lipscomb, Acc. Chem. Res. 22 (1989) 62.
[7] B.M. Britt, W.L. Peticolas, J. Am. Chem. Soc. 114 (1992) 5295.
[8] J. Suh, W. Cho, S. Chung, J. Am. Chem. Soc. 107 (1985) 4530.
[9] S.K. Burley, P.R. David, A. Taylor, W.N. Lipscomb, Proc. Natl. Acad. Sci. USA 87 (1990) 6878.
[10] S.K. Burley, P.R. David, R.M. Sweet, A. Taylor, W.N. Lipscomb, J. Mol. Biol. 224 (1992) 113.
[11] N. Sträter, W.N. Lipscomb, Biochemistry 34 (1995) 9200.
[12] N. Sträter, W.N. Lipscomb, Biochemistry 34 (1995) 14792.
[13] E.E. Kim, H.W. Wyckoff, J. Mol. Biol. 218 (1991) 449.
[14] J.E. Butler-Ransohoff, S.E. Rokita, D.A. Kendall, J.A. Banzon, K.S. Carano, E. Kaiser, A.R. Matlin, J. Org. Chem. 57 (1992) 142.
[15] N. Sträter, T. Klabunde, P. Tucker, H. Witzel, B. Krebs, Science 268 (1995) 1489.

[16] T. Klabunde, N. Sträter, H. Witzel, B. krebs, J. Mol. Biol. 259 (1996) 737.
[17] J.H. Goldberg, Y. Huang, P. Kwon, A.C. Greengard, J. Nairna, J. Kuriyan, Nature 376 (1995) 745.
[18] Y. Chu, E.Y. Lee, K.K. Schlender, J. Biol. Chem. 271 (1996) 2574.
[19] E. Jabri, M.B. Carr, R.P. Hausinger, P.A. Karplus, Science 268 (1995) 1000.
[20] P.A. Karplus, M.A. Pearson, R.P. Hausinger, Acc. Chem. Res. 30 (1997) 330.
[21] T. Yamana, Y. Mizukami, A. Tsuji, Y. Yasuda, K. Masuda, Chem. Pharm. Bull. 20 (1972) 881.
[22] N.H. Williams, J. Chin, J. Chem. Soc. Chem. Commun. (1996) 131.
[23] J. Chin, B. Banaszczyk, V. Jubian, X. Zou, J. Am. Chem. Soc. 111 (1989) 186.
[24] M. Komiyama, J. Biochem. 118 (1995) 665.
[25] J.K. Bashkin, in: K.D. Karlin, Z. Tyeklár (Eds.), Bioinorganic Chemistry of Copper, Chapman & Hall, New York, 1993, pp. 132–139.
[26] B. Meunier, DNA and RNA Cleavers and Chemotherapy of Cancer or Viral Diseases, Kluwer Academic, Dordrecht, 1996.
[27] R. Häner, J. Hall, Antisense Nucleic Acid Drug Dev. 7 (1997) 423.
[28] Many examples are given in: R. Breslow, Acc. Chem. Res. 28 (1995) 146.
[29] K. Shishido, N. Habuka, Biochem. Biophys. Acta 884 (1986) 215.
[30] For a critical discussion on the use of nitrophenyl substrates see: (a) F.M. Menger, M. Ladika, J. Am. Chem. Soc. 109 (1987) 3145. (b) R. Breslow, S. Singh, Bioorg. Chem. 16 (1988) 408.
[31] E. Kövári, R. Krämer, Chem. Ber. 127 (1994) 2151.
[32] E. Kövari, J. Heitker, R. Krämer, J. Chem. Soc. Chem. Commun. (1995) 1205.
[33] E. Kövari, R. Krämer, J. Am. Chem. Soc. 118 (1996) 12704.
[34] R. Krämer, GIT Fachz. Lab. 40 (1996) 1170.
[35] E. Kövari, R. Krämer, Z. Naturforsch. 49b (1994) 1324.
[36] R. Ott, R. Krämer, Angew. Chem. Int. Ed. 37 (1998) 1957.
[37] R. Breslow, D. Berger, D.L. Huang, J. Am. Chem. Soc. 112 (1990) 3686.
[38] F. Chu, J. Smith, V.M. Lynch, E.V. Anslyn, Inorg. Chem. 34 (1995) 5689.
[39] S. Liu, A.D. Hamilton, Tetrahedron Lett. 38 (1997) 1107.
[40] E. Kimura, M. Kodama, T. Koike, M. Shiro, J. Am. Chem. Soc. 117 (1995) 8304.
[41] M.J. Young, D. Wahnon, R.C. Hynes, J. Chin, J. Am. Chem. Soc. 117 (1995) 9441.
[42] J.R. Morrow, K. Aures, D. Epstein, J. Chem. Soc. Chem. Commun. (1995) 2431.
[43] H.-J. Schneider, J. Rammo, H. Rettich, Angew. Chem. Int. Ed. Engl. 32 (1993) 1716.
[44] J. Rammo, H. Rettich, H.-J. Schneider, J. Chem. Soc. Chem. Commun. (1996) 105.
[45] A. Schepartz, R. Breslow, J. Am. Chem. Soc. 109 (1987) 1814.
[46] J. Suh, Acc. Chem. Res. 25 (1992) 273.
[47] J. Suh, T.H. Park, B.K. Hwang, J. Am. Chem. Soc. 114 (1992) 5141.
[48] J. Suh, Bioorg. Chem. 18 (1990) 345.
[49] J. Suh, B.K. Hwang, Y.H. Koh, Bioorg. Chem. 18 (1990) 207.