1

Introduction

Enzymes are an essential part of life which can be found in diverse organisms like bacteria, fungi, plants and mammals. They are remarkable catalysts because they are able to operate very regulated, efficient as well as substrate-, regio- and stereospecific. Additionally, all enzymatic processes take place under physiological conditions which means ambient temperature, mostly neutral pH and normal pressure. For example processes like the conversion of water to dioxygen (photosystem I and II), the cleavage of the dinitrogen bond to form ammonia (nitrogenase) or the conversion of urea to carbondioxide and ammonia (urease) are not that fast under physiological conditions without enzymes.
What is the reason that enyzmes operate in such an efficient way? The answer can be found in their construction. Enzymes consist of a metallated active site and a huge protein backbone. In the active site the metal ion(s) is/are ligated by a number of various amino residues which forms the primary coordination sphere. Close to the primary coordination sphere can be found the secondary coordination sphere

which is responsible for the formation of non–coordinating interactions. These non–coordinating interactions are hydrogen bondings, electrostatic, hydrophobic effects and van–der–Waals forces, which stabilise highly reactive intermediates or build up a channel for proton and/or electron transfers. [1–3]

The scientific challenge is to create bioinspired model complexes which emulate the structural and functional properties of a native enzyme. Structural models are mostly helpful to estimate the structure of the enzymes active site (speculative models). The effect on spectroscopic and catalytic properties of the metal ion can be investigated with the design of different ligand systems (corroborative models). Functional model complexes are developed for particular applications. These applications can be for instance for catalysis or medicine, whereas the structural resemblance of functional motifs can be minimal in certain cases. However, mimicking the entire enzyme is proven to be very difficult. [4] Therefore, mainly low-molecular weight molecules are developed to mimic the active site. They are mostly easier to handle which is related to cleaning steps as well as sensitivity and they can be very helpful to study the catalytic mechanism by for instance isolating a substrate to metal bonded intermediate.

In this work two different kinds of ligand systems and their corresponding complexes were synthesised to emulate diverse enzyme active sites. The first part deals with the preparation of small mononuclear zinc(II) and copper(II) complexes which were investigated towards their catalytic properties to degrade organophosphates. The second part deals with the synthesis of more sophisticated ligands and their corresponding dinuclear nickel(II), zinc(II) and copper(I/II) complexes. By means of additional functional groups like amides and amines attached to the chelating sidearms, the ligands are able to illustrate an *intra*molecular hydrogen bonding network. These hydrogen bonds either assist the conversion of substrates and improve the catalytic activity or they are able to stabilise highly reactive intermediates which can be isolated and characterised.

2

Phosphatase Models

2.1. Zinc and Its Biological Role

Zinc is after iron the second most abundant 3d metal in biology and the human body (2 – 3 g). [5] But the chemical properties of zinc(II) differ strongly from other transition metals like iron or copper. Due to its completely filled d orbitals the spectroscopic detection of zinc(II) ions is rather limited as it neither displays several redox states, nor absorbs in the UV/vis spectral regions. [6] Fortunately, in some cases zinc(II) can be replaced by other metal ions (cobalt(II) or copper(II)) without destroying the catalytic activity of the enzyme. [7,8] And due to zinc(II) forms diamagnetic complexes, these complexes can be excellent analysed by NMR technique.
Zinc(II) is part of the borderline metals and can be coordinated by both the sulfur atom of cysteine and nitrogen atom of histidine (soft character) or oxygen atom of aspartate as well as glutamate (hard character). This lack of an energetic barrier makes zinc(II) an excellent

metal for a rapid ligand exchange and a flexible coordination geometry in catalytic cycles (e.g. from a tetra- to a penta-coordination).[9–11] Furthermore, specifically zinc(II) can coordinate in different ways to the named amino acids. It can be ligated to the N_δ or N_ε nitrogen atom of histidine, to the $O_{\varepsilon 1}$ or $O_{\varepsilon 2}$ (*syn* or *anti*) oxygen atom of glutamate/aspartate and to the sulphur atom of cysteine either in a monodentate or bridging fashion (figure 2.1).[10] More rarely zinc(II) binds to carboxamide and phenolate functional groups as it is represented in asparagine as well as glutamine[12,13] and tyrosine,[14] respectively.

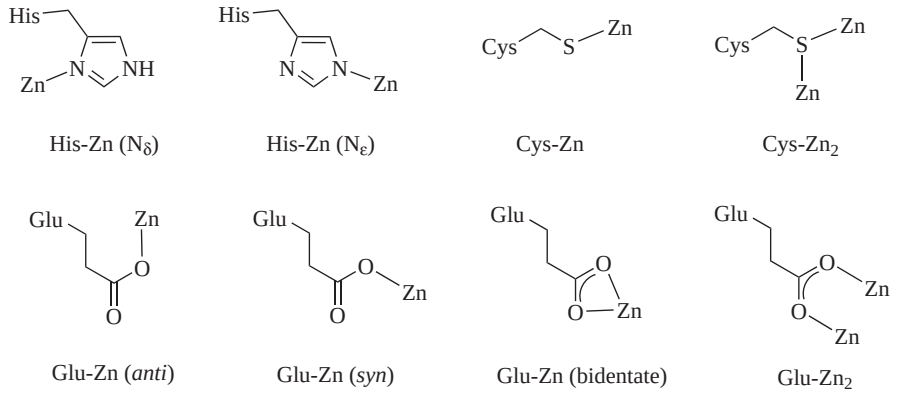


Figure 2.1.: Zinc–amino acid residue binding modes.[10]

In enzymes zinc(II) contains mostly one variable coordination position which is mainly occupied by a water molecule. This water molecule can be either displaced easily by a substrate/inhibitor or will be transformed into a catalytic active species (hydroxide ion) by lowering the pK_a value. The deprotonation can occur at neutral pH and is activated by the zinc(II) ion due to its strong Lewis acidity (e.g. in carbonic anhydrase[15]). For less Lewis acidic zinc(II) centres the activation occurs through interaction with an adjacent base (e.g. in carboxypeptidase[16]).[10] Zinc is found as an active site metal ion in all six enzyme classes whereas the hydrolases are the most common ones. Moreover, its coordination environment has been defined into four main categories: catalytic, cocatalytic (enhancing activity), structural and interface.[17,18]

2.2. Enzymes Cleaving Phosphate Esters

Phosphatases belong to the enzyme class of hydrolases. Hydrolases are capable of catalysing the cleavage of phosphate ester bonds. Based on their substrate specificity phosphoesterase enzymes are divided into three groups: phosphomonoesterases, phosphodiesterases and phosphotriesterases. In many of the phosphatases, the zinc(II) ion is found in the active site as shown in table 2.1. The most important and best characterised phosphatases are emphasised and will be introduced in the following sections.

Table 2.1.: The phosphoesterase enzymes belong to the enzyme class of hydrolases and can be divided into three groups depending on their catalytic properties. [19]

Enzyme class	Enzyme	Metals
Phosphomonoesterase	*Alkaline Phosphatase*	Zn^{2+}, 1 Mg^{2+}
	Purple Acid Phosphatase	Fe^{3+}, 1 Zn^{2+}
	Protein Phosphatase 1	Fe^{3+}, 1 Zn^{2+}
	Calcineurin	Fe^{3+}, 1 Zn^{2+}
	Inositol Monophosphatase	M^{2+}
	Fructose 1,6-diphosphatase	M^{2+}
Phosphodiesterase	*Nuclease P1*	Zn^{2+}
	Phospholipase C	Zn^{2+}
	Klenow Fragment	M^{2+}
Phosphotriesterase	*PTE* from *Pseudomonas diminuata*	Zn^{2+}

2.2.1. Phosphomonoesterase

Alkaline Phosphatase

Alkaline phosphatases (AP) hydrolyse phosphate monoesters nonspecifically under both acidic and basic conditions. [20] But the optimal activity is found around pH 8.0, hence the name. [11] The best studied AP is that from *E.coli* which consists of two zinc(II) ions and one magnesium(II) ion in each subunit of a homodimeric protein. The two zinc(II) ions (1 and 2) represent the catalytic active centre whereas the magnesium(II) ion is not catalytically active. The role of the magnesium(II) ion is generally believed to be ancillary. [21] But nonetheless, it serves to enhance the enzymatic activity because a replacement of the magnesium as a mutating enzyme caused a drastically lowering in activity of the Zn2(II) ion. [22] This effect is much lower for bacteria organisms than for mammals. [19]

The two zinc(II) ions are coordinated by histidine and aspartate residues. In the absence of a substrate the free coordination sites are occupied by water molecules. The Zn2(II) ion is also located close to a serine(102) which has a significant function in the catalytic mechanism. [20] Experiments show that the replacement of this serine(102) residue for alanine or leucine affects an increase in activity. [23] The magnesium(II) ion is surrounded in a slightly distorted octahedral coordination sphere comprised of glutamate(332), threonine(155), aspartate(51) and three water molecules. The aspartate(51) is already linked to the Zn2(II) ion. [20] The zinc(II) ions are 4 Å apart. The magnesium(II) ion is located 5 – 7 Å from the dizinc(II) centre (figure 2.2). [24]

The catalytic mechanism is presented in scheme 2.1. The hydrolysis starts with the replacement of the water molecules and the ligation of the phosphate to the dizinc(II) core. [26] The ester function is arranged near the Zn2(II) ion and the serine(102). The overall bidentate enzyme–substrate intermediate is stabilised by hydrogen bondings to an arginine(166) residue. The active nucleophile is believed to be

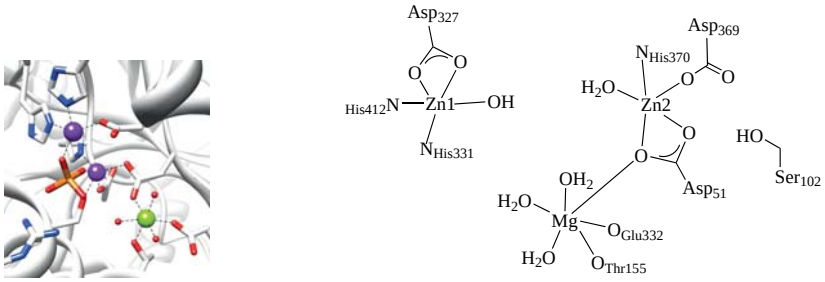
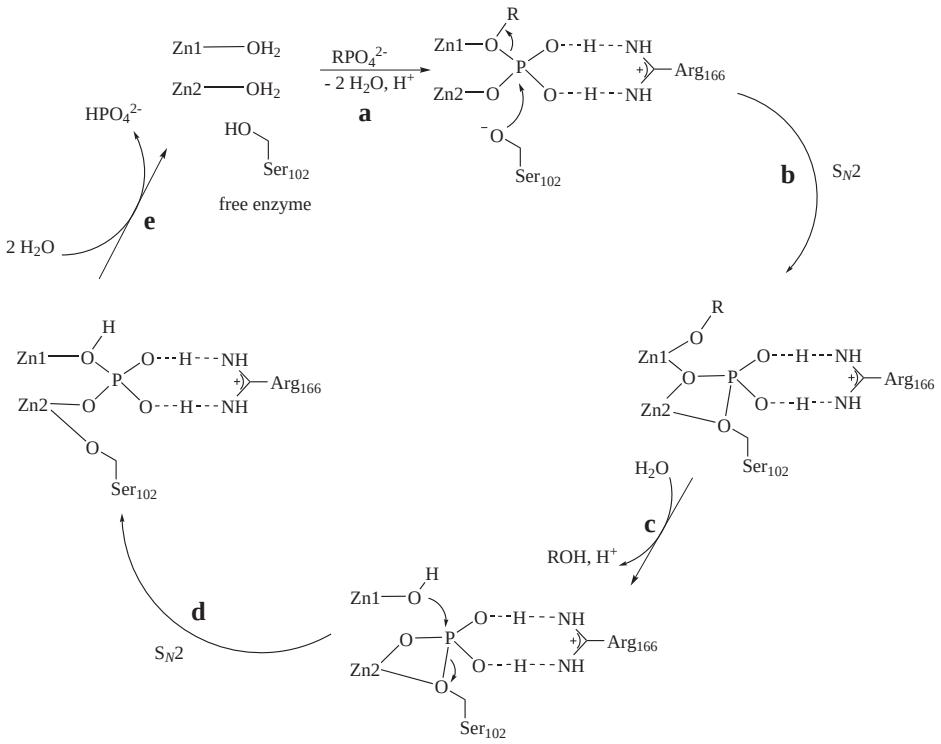


Figure 2.2.: Crystal structure of the active site of AP from *E.coli* with coordinated phosphate (violet: zinc(II); green: mangnesium(II); orange: phosphorus; left, PDB Code: 1ALK [25]) and chemical structure (right) of the active site without the coordination of a substrate.

the deprotonated serine(102) (**a**). Besides the nucleophilic attack the anionic serine(102) residue is probably stabilised by an interaction with the Zn2(II) ion. The pK_a of the serine(102) has been reported to be $\leq$ 5.5 in the free enzyme. [27] It has been suggested that the deprotonation of the serine(102) is regulated by the magnesium(II) ion. It provides an additional metal–bond water in the vicinity of serine(102). This metal bonded water can function as a general acid or base, depending on the catalytic conditions.
After the S_N2 reaction the alkoxide is cleaved (**b**) and is displaced by a water molecule (**c**). This water molecule is deprotonated and functions as a nucleophile which can attack the phosphoseryl intermediate (**d**, characterised by a solid state structure [28]). A consequence of this so called "ping-pong" mechanism is the retention of the absolute configuration at the phosphorus atom, a fact confirmed by isotope-labelling (^{18}O) experiments [29]. Finally, the hydrolysed phosphate product is released from the dinuclear zinc(II) centre, the free coordination positions are occupied by water molecules and the free enzyme is regenerated (**e**). [19,21]



Scheme 2.1: Proposed reaction mechanism for the hydrolysis of a phosphomonoester by the enzyme AP. The enzyme structure is simplified for a better overview. [19]

Purple Acid Phosphatase

Purple acid phosphatases (PAP) are counterparts to APs. They can be found in mammals, plants, fungi as well as in bacteria. They hydrolyse activated phosphoric acid esters and anhydrides like ATP at acidic to neutral pH (4 – 7). [30,31] In general the enzyme has a binuclear heterovalent catalytic centre of one iron(III) ion and a metal ion with a charge of two which can be either zinc(II), manganese(II) or iron(II). [32]

The metal ions are surrounded by seven invariant amino acid residues. In the resting state, the iron(III) ion is bound to a tyrosine(167), a histidine(325) and an aspartate(135), while the metal(II) ion is ligated by an asparagine(201) and two histidine(286, 323) residues. Both metal ions are linked to a bridging monodentate carboxylate ion of an as-

partate(164) side chain and a μ-OH group. The coordination spheres of the five- and six-coordinated iron(III) and metal(II) ions are completed by a terminal water ligand (d = 3.1 Å, [12] figure 2.3). [33,34]

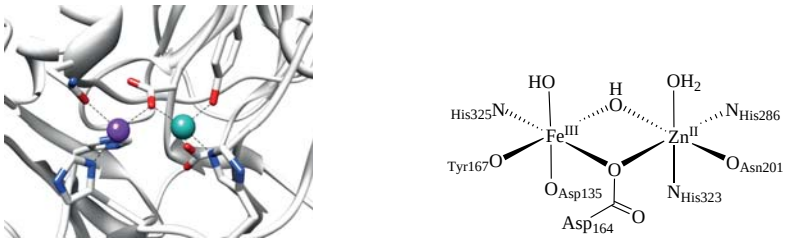


Figure 2.3.: Crystal structure of the active site of PAP isolated from kidney bean (violet: zinc(II), blue: iron(III); left, PDB Code: 1KBP [31]) and chemical structure (right).

While the PAPs in plants contain a redox-inert Fe^{III}/M^{II} (M = Zn or Mn) core in the catalytic unit, the mammalian PAPs consist of a redox-active centre $Fe^{III}/Fe^{II/III}$, in which only the mixed-valent form is catalytically active. Due to the low redox potential (ca. 340 mV) the redox-active centre can be easily and reversibly oxidised to the inactive diferric form. Both, however, exhibit the characteristic purple colour which arises from a ligand-to-metal-charge transfer (LMCT) between the tyrosinate ligand and the iron(III) ion (λ_{max} = 510 – 560 nm). It has been shown that PAPs are active towards diphosphate esters. [32] Furthermore, it is also discussed that the diiron enzyme from mammalians can activate dioxygen in addition to the hydrolytic function. [35]

Despite the detailed structure description the catalytic mechanism is not yet fully understood. Two different mechanistic pathways have been proposed concerning the substrate binding mode and the following nucleophilic attack. KREBS *et al.* suggest a mechanism for red kidney bean PAP. The substrate binds in a monodentate fashion to the metal(II) ion, whereas the iron(III) ion provides a terminally bound hydroxide ion as nucleophile at acidic pH (figure 2.4**a**). [31] An alternative catalytic pathway is proposed for pig and sweet potato PAPs in which the substrate forms a μ-1,3-phosphate–intermediate

bridge. A bridging hydroxo or oxo group carries out the nucleophilic attack (figure 2.4**b**).[36] Recent studies have indicated that these two flexible mechanistic strategies depend on the metal ion composition, second coordination sphere and the substrate itself.[33]

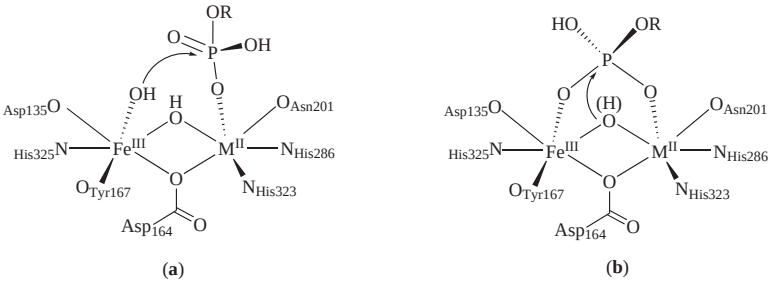


Figure 2.4.: Two different proposed phosphate intermediates in the catalytic hydrolysis of PAPs.
(**a**) Binding of a monodentate phosphate and attack of a terminal bound hydroxide ion.[31]
(**b**) Forming of a μ-1,3-phosphate–intermediate bridge and conducting the nucleophilic attack by a bridging hydroxo or oxo group.[36]

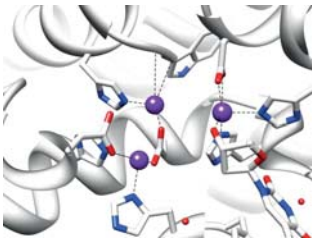
2.2.2. Phosphodiesterase

P1 Nuclease

The P1 Nuclease (P1) was isolated from *Penicillium citrinum* and features a trinuclear zinc(II) site in the active centre. P1 is a phosphodiesterase which degrades single-stranded RNA and DNA. It cleaves the bond between the 3'hydroxyl and 5'phosphoryl group with inversion of the configuration at the phosphorus atom. At the same time the enzyme acts as a phosphomonoesterase by removing the 3'terminal phosphate group.[37,38]

P1 features a trinuclear zinc(II) ion core. All of these zinc(II) ions have three *O*– and two *N*–donor ligands which are bound in a trigonal bipyramidal geometry (figure 2.5). The Zn1(II) and Zn3(II) ions are 3.2 Å apart from each other and are bridged by the aspartate(120) residue and a water molecule/hydroxide ion. The Zn2(II) ion is placed

5.8 Å apart from the Zn1(II) ion and 4.7 Å from the Zn3(II) ion. The Zn2(II) ion binds to two additional water molecules besides the two histidine(126,149) and one aspartate(153) side chains. The overall protein structure is almost the same as in phospholipase C. They only differ in the binding of a glutamate instead of an aspartate to the Zn2(II) ion. [19,37,39]



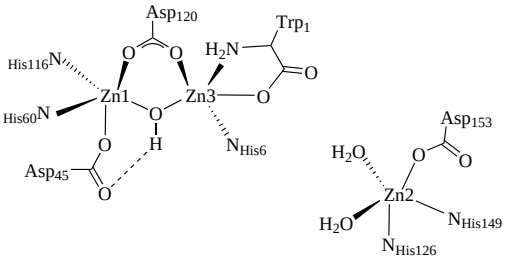
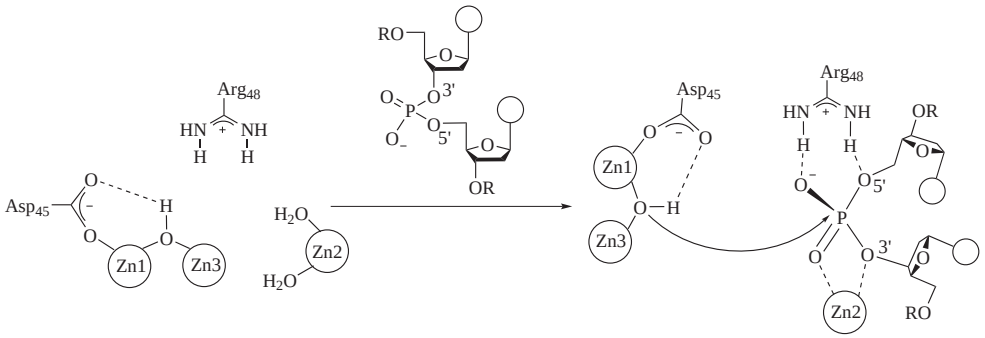
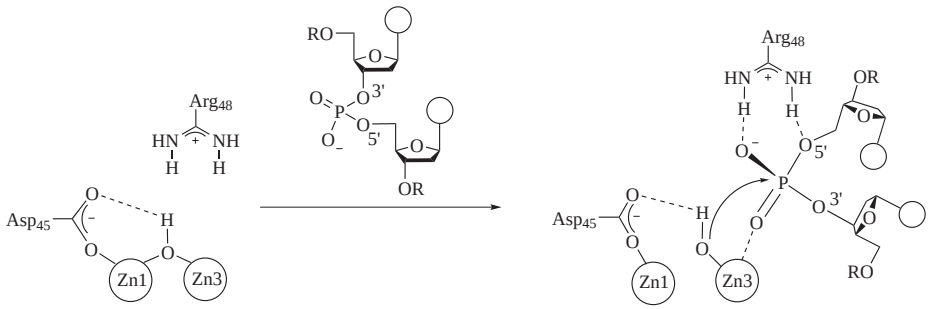


Figure 2.5.: Crystal structure of the active site of the P1 enzyme without water molecules (violet: zinc(II), left, PDB Code: 1AK0 [40]) and chemical structure (right).

Based on crystallographic data, two mechanism pathways are discussed. Both mechanisms involve a nucleophilic attack of an activated water molecule bonded on the zinc(II) site and a stabilisation of the penta-coordinated transition state by arginine(48) via the formation of a hydrogen bonding network. [40] In one mechanism the μ-OH bridging unit is considered to be the active nucleophile. Computational evidence for a catalytic bridging hydroxide ion is reported in literature (scheme 2.2). [41] However, this hydroxide ion exhibits a rather low nucleophilicity in this tight bridging state. An alternative mechanism suggests that a shift of a terminal hydroxide ion occurs which then attacks the ligated substrate (scheme 2.3). [42]



Scheme 2.2: Proposed mechanism in P1 with a catalytic μ-OH bridging ion. [41]



Scheme 2.3: The alternative mechanism exhibits a nucleophilic terminal hydroxide ion. [42]

2.2.3. Phosphotriesterase

Phosphotriesterase (PTE), isolated from *Pseudomonas diminuate*, catalyses the hydrolysis of different organophosphates (OPs), but to date there is no natural substrate known which is hydrolysed by the PTEs. [19] OPs are among the most toxic compounds that have been chemically synthesised [43–45] and are used in a broad spectrum as plasticiers, petroleum additives, insecticides (e.g. paraoxon, parathion, figure 2.6 (top)) and chemical warfare agents (CW agents, e.g. sarin, soman, VX, figure 2.6 (bottom)). [46,47] Finally, the resistance of some insects to insecticides and the degradation of them by soil microbes led to the discovery of PTEs. [48–50]

The strong toxicity of the OPs for human bodies and animals arises from the irreversible binding on the acetylcholinesterase (AChE) en-

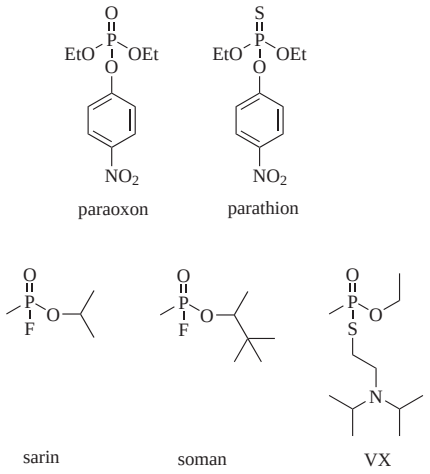
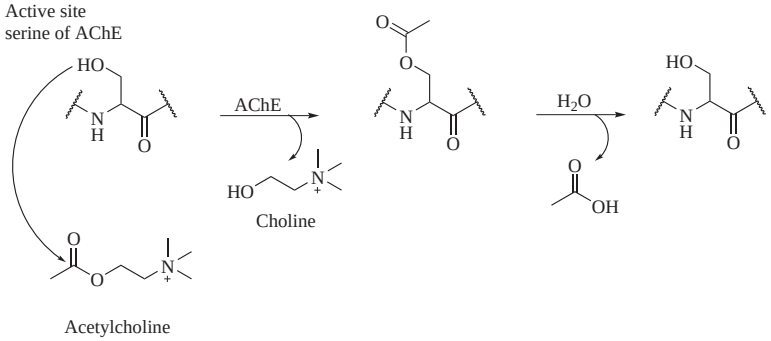


Figure 2.6.: Examples for insecticides (above) and CW agents (bottom). [51]

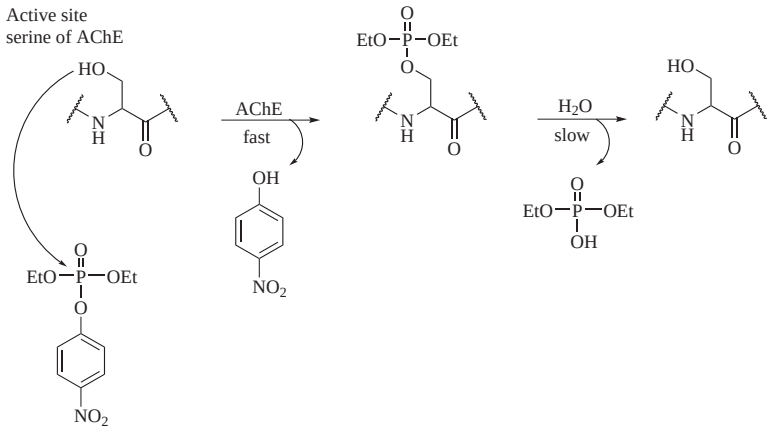
zyme. [52,53] AChE hydrolyses acetylcholine, a neurotransmitter that relays nerve impulses to muscles and other organs. [44,45] The active centre of the AChE is a serine residue where the hydrolysis occurs within two steps. The first step is the nucleophilic attack on the carbonyl–carbon atom of acetylcholine to form a covalent acetyl–enzyme intermediate. Choline is released from the active site. The regeneration takes place in the second step via a hydrolytic attack by water and the release of acetate (scheme 2.4). [51]



Scheme 2.4: Catalytic hydrolysis of acetylcholine by the enzyme AChE. [51]

The OPs react in the same way with the active centre of the AChE. The formation of the covalent bond to the serine residue in the active site is very fast whereas the second step which stops the enzyme

from functioning is quite slow (scheme 2.5). The successive build up of acetylcholine blocks cholinergic nerve impulses and leads to paralysis, suffocation and death. [44,45,54]



Scheme 2.5: Inhibition reaction of AChE by paraoxon. [51]

For that reason the concern in the decomposition of OPs to non–toxic materials for environmental protection increases continuously. In particular, the degradation of CW agents is of great interest. [55] The increased sophisticated synthesis of nerve gases dates back to World War II when compounds like tabun, sarin and cyclosarin (G-types) were developed and synthesised. They were not used during World War II but in several other military conflicts. In the 1950s, the V-type nerve agents, which are more toxic and persistent than the G-agents, were developed. The level of world stockpiles CW agents are significant high all over the world. That is why, in 1997, the United States ratified the Chemical Weapons Convention (CWC) Treaty (drafted 1992; signed 1993; effective 1997), which bans the possession as well as production of CW agents and requires that the signatory nations destroy any CW agent stockpiles. [52,56,57]
The first decontaminations were bleaching powders and solutions. Because of several disadvantages like corrosion problems and quantity decrease with long storage time, better and more effective decontaminations were developed over the years. [58] These effective decontaminations include hydrolysis under acidic, neutral and basic condi-

tions, nucleophilic–assisted substitution, surface chemistry, the use of ionic liquids, and synthesis of model complexes based on the PTE enzyme.[44,52,56,58,59] Furthermore, other methods were developed which cannot decompose CW agents but which are able to detect CW agents or degradation products. These methods are based on colorimetric dyes or chemiluminescence reaction.[56,60]

As aforementioned the natural enzymes are these which contain a dizinc(II) core. Both metal ions are ligated in a distorted trigonal bipyramidal geometry by five amino residues. These residues are histidine(57, 55) and a monodentate coordinated aspartate(301) for Zn_α, whereas the second zinc(II) ion (Zn_β) is surrounded by two histidine(230, 201) residues and one water molecule. This water molecule can be replaced easily by a substrate. Moreover, a lysine(169) is provided as carbamate bridge between both zinc(II) ions as well as a second water or hydroxide ion. The distance between Zn_α and Zn_β is 3.4 Å and the dinuclear centre is surrounded by a hydrophobic environment (figure 2.7). The ligation geometry resembles closely the dinuclear nickel(II) centre observed in the urease enzyme.[61,62]

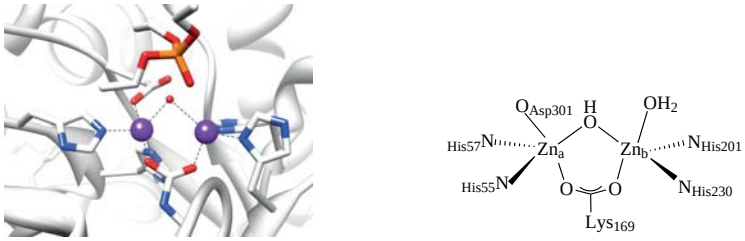


Figure 2.7.: Crystal structre of the *Pseudomonas diminuta* PTE enzyme with a non–coordinated OP (violet: zinc(II), orange: phosphorus, left, PDB Code: 1EYW [63]) and chemical structure (right).

Three binding pockets were identified by X-ray crystallography. These subunits are described as small, large and leaving group pockets and are capable of hydrolysing particular substrates (with P–O, P–F, P–CN, P–S bonds [48,64]).[61,63,65,66] The purified protein shows a remarkable catalytic activity towards the hydrolysis of the commer-