Why does nature use zinc–a personal view†

Heinrich Vahrenkamp

In this perspective some results from the work by the author's research group on the coordination chemistry of zinc are presented. They are selected so as to highlight the principles which, in the opinion of the author, make zinc unique in its ability to function as the catalytic center for an ever-increasing number of biological processes. In essence, the "non-properties" of zinc are the basis of its success: no redox chemistry, no ligand field effects, no typical coordination numbers or geometries, no stability or inertness of its complexes, no typical "hard" or "soft" characteristics. The chemistry presented here is centered around the "Freiburg Enzyme Model", the pyrazolylborate-ligated zinc-hydroxide complex. The topics discussed in detail include the zinc–water combination, the non-similarity between the Zn–OH and the Zn–SH, Zn–OR or Zn–SR complexes, and the functionalization of CO_2 by zinc-bound nucleophiles. The value of structure correlation analysis for the elucidation of mechanistic details is demonstrated, and the first examples of catalysis by the TpZn–OH complexes are presented.

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† This perspective is dedicated to the memory of Swiatoslaw ("Jerry") Trofimenko whose seminal work and whose generous help have made our work possible.

Heinrich Vahrenkamp

Heinrich Vahrenkamp, born in 1940, received his doctoral degree at the Universität München in 1967 under the supervision of Heinrich Nöth with a thesis on boron chemistry. During his postdoctoral years, at the University of Wisconsin in the group of Larry Dahl, he mutated to a transition metal chemist. His Habilitation work, finished in 1972 at the Univerität München, was on organometallic Lewis bases. At the age of 32 he became the then youngest professor of chemistry in Germany, holding a chair of inorganic chemistry at the Universität Freiburg, where he has stayed ever since. In Freiburg he developed systematic cluster chemistry for 20 years which, in the nineties, evolved into the study of cyanide-bridged chain-like oligonuclear complexes. Starting in the late eighties, the main and finally sole focus of his work became the coordination chemistry of zinc related to the biological functions of zinc. Out of this resulted the "Freiburg Enzyme Model". Having retired in 2005, he now enjoys life as the Chairman of the Wissenschaftliche Gesellschaft, the leading local funding organization supporting Freiburg University, and as a visiting professor in exotic countries.

Introduction

No other metal has as many functions in living organisms as the metal zinc. In one of the early reviews on its biochemistry[1] Vallee noted that it occurs in all six groups of the IUBMB enzyme classification. The number of enzymes known to contain zinc in the active center now exceeds 1000.[2] In mid 2007 the Brookhaven protein data bank lists some 40 000 structures of proteins, out of which almost 5000 contain zinc, and it has been found that 3% of the human genome contain the code for zinc finger proteins.[3] All this despite the fact that the zinc ion neither possesses colour, magnetism or redox properties, nor provides stability or inertness for its complexes, which for generations has made zinc the "boring element" in teaching and research, with the result that authors cannot even agree on where to place it in the textbooks.

In the majority of cases the rôle of zinc ions in proteins is structural, comparable to that of hydrogen bonds. The famous examples for this are zinc fingers. However, the remaining number of cases with zinc in a functional rôle, *i.e.* zinc enzymes, still exceeds the corresponding number for any other metal. While, as mentioned above, zinc enzymes occur in all enzyme classes, their vast majority is of the hydrolytic type, *i.e.* responsible for the making and breaking of carboxamide, phosphate and carboxylic ester bonds and for the handling of CO_2 in aqueous media. This is the realm of zinc in biology where it is active in good and evil things alike, ranging from the strengthening of the immune system to the spreading of antibiotic-resistant bacteria, or from the fatal action of snake venoms to the cosmetic illusion of a Botox treatment. It is no exaggeration when one states that zinc is everywhere.

Considering this background the question "Why does Nature use zinc?" need not be uttered too loudly. Many people have tackled it, and my answers given here are not essentially new. I was fascinated by zinc in life processes even before the term "bioinorganic chemistry" was established: almost 40 years ago I wrote a popular science article "Metals in Life Processes" in which zinc constitutes an important part.[4] I dared not enter the field of bio-related zinc chemistry then; it seemed to be too risky at the

beginning of a career. But after success was established I jumped into it, beginning with the article "Zinc, a boring element?" in the same popular science journal,[5] a year before the first coworker started with zinc in the lab, and in the last 10 years before my retirement nothing but zinc chemistry was investigated by my research group.

I have avoided as much as possible calling myself a bioinorganic chemist. We have worked on the coordination chemistry of zinc related to its biological functions. This perspective lists some of our results which in my opinion are relevant in this context. They may contribute to the answering of the title question which, of course, like all "Why" questions in the life sciences will never find a final answer.

The non-properties

The uniqueness of zinc is well-hidden. Unlike iron, as in the blood or in the meat, we do not see it. Unlike cobalt, nickel, copper or cadmium we can hardly poison ourselves with its compounds. It is only appropriate in this scenario that one has a hard time finding zinc in the textbooks. Thinking about this, one can come to the conclusion that when there are no characteristic features leading the way to the unique properties of zinc in biology, their absence may be the clue. *Viz*, the non-properties of zinc are the secret of its success.

Transition metal or not?

Whether zinc with its filled d shell is a transition metal or not may be a semantic question, but of course its properties resulting from the filled d shell are essential. Some of them will be discussed here. Others, like the lack of colour, magnetism or unpaired electrons, which exclude zinc from the biological realm of manganese, iron or cobalt, bear no relevance to its own functionality.

A rather trivial consequence of the electronic configuration of zinc and hence its chemical properties is its bioavailability. While the omnipresence of iron is easily explained by the fact that it is the fourth-most abundant of all elements, zinc together with manganese, chromium, nickel and copper belongs to the rarer elements (0.1–0.01% of the lithosphere). However, unlike these partners it does not form a sulfide, oxide, carbonate or silicate of extremely low solubility. As a result it is present in non-negligible concentrations in all waters, sometimes to the extent that the strict drinking water or wastewater regulations give reason for concern.

Less trivial are the consequences of the fact that there are no ligand field effects in the chemistry of zinc. Notably, zinc complexes lack inertness and typical geometries. In terms of reactivity that means low activation barriers, the foremost prerequisite for efficient catalysis. Together with the generally low thermodynamic stability of zinc complexes these properties can make preparative zinc chemistry a rather unpleasant business. But in its biology they make zinc what it is: available, mobile and versatile.

What about the other divalent metals?

Zinc is not the only divalent metal that should be able to perform its biological functions. Magnesium is often mentioned because its radius is not too different from that of zinc, and divalent cobalt shares its coordinative flexibility. However, recalling some basic knowledge eliminates these candidates easily and definitely.

Much effort has been invested in this respect into the substitution of zinc by cobalt in its enzymes,[6] enabling the use of optical spectroscopy to gain information about the catalytic center. Amazingly, some of the cobalt-substituted enzymes do their job better than the native zinc enzymes. Yet, Nature does not use cobalt here, for the simple reason that in a protein environment and in the presence of air cobalt will rapidly become trivalent-inert, stable, dead.

In the past the substitution of zinc in enzymes by cadmium was also attempted, in an effort to take advantage of ^{113}Cd NMR spectroscopy in gaining structural and mechanistic information.[7] These efforts were abandoned, not only because its toxicity eliminates cadmium anyway, but because it became too obvious that cadmium and zinc have essentially nothing in common in terms of ligand preferences, coordination numbers or stabilities. Even if cadmium finds the position originally occupied by zinc, it cannot behave like the latter there.

The question whether calcium or magnesium could play the rôles of zinc cannot be negated in such simple terms. The intuitive answer is that they are too typical as main group elements, *i.e.* they do not prefer the soft nitrogen and sulfur environments of the zinc sites, and that their complexes with nitrogen-containing ligands are of even lower stability and higher lability than those of zinc. Yet, considering that they are much more abundant in living organisms than zinc, it is an attractive question as to why evolution did not opt for them when designing the hydrolytic enzymes.

Which coordination number?

A typical textbook entry for each metal is the table of coordination numbers and geometries. This is where for zinc we find a classical textbook error: the $Zn(NH_3)_6^{2+}$ complex. It has to exist because every decent metal forms a hexammine complex, and as such it appears in about a dozen older publications. But zinc is stubborn, and under ordinary conditions it only forms the $Zn(NH_3)_4^{2+}$ complex. Only very recently and rather exotically has a compound containing the $Zn(NH_3)_6$ cation been isolated, which persists only at −100 °C: by using metallic zinc to reduce fullerene in liquid ammonia, Jansen *et al*. obtained and structurally characterized the hexammine zinc salt of dianionic C_{60}.[8]

This story is told here to underline the "flexibility" of zinc coordination. Indeed the variety of coordination numbers or geometries for zinc exceeds that of all other metals. On one side the linear twofold coordination, as in the zinc dialkyls, needs no steric protection. On the other hand the archetypal zinc blende structure nourishes the illusion that zinc is best accommodated in a symmetrical tetrahedral environment. Yet the abundance of structural data now tells us that a symmetrical coordination is the exception rather than the rule for zinc complexes. This has been handled with much detail for ZnL_5 species,[9] and the identification of the species present in aqueous solutions of $ZnCl_2$ has been a real challenge.[10]

Everyone active in the coordination chemistry of zinc has stumbled upon these types of problems. We did it in our pyrazolylborate zinc chemistry. The 3,5-disubstituted pyrazolylborates, once called tetrahedral enforcers by Trofimenko,[11] will adapt to necessity too in terms of coordination numbers. As Scheme 1 shows, they can form 4-, 5-, or 6-coordinate zinc complexes depending on the
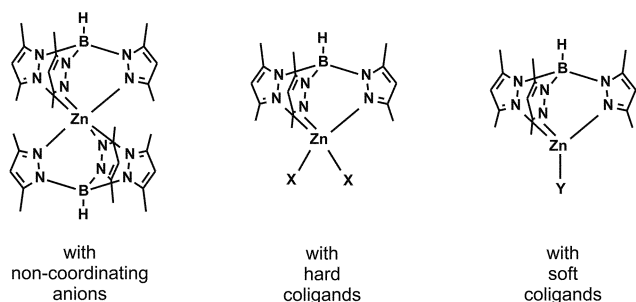
Scheme 1 Pyrazolylborate zinc complexes with 4-, 5-, and 6-coordinated zinc.

counteranions present,[12] an observation that will be discussed in detail below.

On the other hand the steric hindrance in the cavity of scorpionate ligands can be made so severe that coordination sites can no longer be occupied. Our example for this is complex **1** with "less than trigonal pyramidal" zinc coordination, *i.e.* the axial-equatorial bond angles are less than 90°.[13]
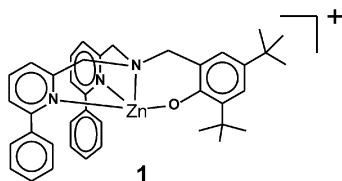
Hard or soft?

Again, no other metal offers so many opportunities for arguments when it comes to the question whether it is hard or soft according to the Pearson nomenclature. At the same time zinc offers the nicest examples for some basic rules in this field, *i.e.* "soft" goes with low coordination numbers and covalency, and "hard" goes with high coordination numbers and ionicity. Examples of the former are the zinc alkyls or the low-coordinate pyrazolylborate zinc complexes in Scheme 1. Likewise in zinc-thiolate or zinc-chloride complexes one rarely finds species with coordination numbers greater than four. On the other hand oxygen-containing ligands impose coordination numbers five and six on zinc, making zincite (ZnO) with the wurtzite structure a misleading exception. Thus there are enough examples for the generally accepted assessment that zinc can be hard *or* soft.

In my opinion this assessment is better formulated as "zinc is *neither* hard *nor* soft" or "is hard *and* soft". The structural biology of zinc underlines this by the extreme dominance of the ZnN_2S_2 coordination (N for histidine, S for cysteinate), the standard motif for structural zinc in enzymes and zinc fingers. The preparative zinc chemist experiences this when using ligands which offer other donors in addition to amine and thiolate. These other donors remain uncoordinated, and ZnL_2 complexes with ligands derived from aminoethanethiolate are commonplace.

The clearest illustration of my proposal that zinc is hard *and* soft is provided by our tripod zinc complexes in Scheme 2.[14–16] Mixed hard and soft ligand environments make for perfectly stable complexes, including even the "non-coordinating" perchlorate ligand. Purely hard or soft ligand environments lead to trouble, making zinc unwilling to accept its most welcome ligand thiolate. In my opinion this is one of the most important "non-properties" governing the biological chemistry of zinc.

Zinc and water

It is a common belief that aqueous solutions of zinc salts are quite acidic, implying that the pK_a of the hydrated zinc cation is rather low. Closer inspection reveals that the former is the case only for concentrated solutions of zinc chloride, and that the pK_a of $Zn(H_2O)_6^{2+}$ is neither low nor easy to determine.[17] This results from its tendency to undergo condensation reactions at pH values above the pK_a, leading to the infamous class of basic zinc salts. The story of $ZnCl_2$ in water is even more complicated, involving a concentration-dependent interplay of condensations, chloride co-ordinations, coordination number changes, and deprotonations.[10] The simplest acidic species in these equilibria is $ZnCl_2(H_2O)_2$, *i.e.* a low-coordinate zinc-aqua complex.

Zinc enzymes in the resting state almost without exception contain zinc-bound water.[18] As Scheme 3 shows, their zinc coordination can be classified in a simple way. In many cases, characteristically in the ADH/CAIMS group, the water molecule only represents the vacant coordination site which during catalysis is occupied by the substrate. More importantly, though, and exclusively in the CA/MMP group, the water ligand is the functional reagent which is deprotonated to become the powerful Zn–OH nucleophile which is responsible for the efficiency of the hydrolytic zinc enzymes. The deprotonation must occur under physiological conditions, *i.e.* near pH 7. Hence the coordination

N$_3$Zn-X

OClO$_3$ — unstable   SC$_2$H$_5$ — stable

S$_3$Zn-X

SC$_2$H$_5$ — unstable   OClO$_3$ — stable
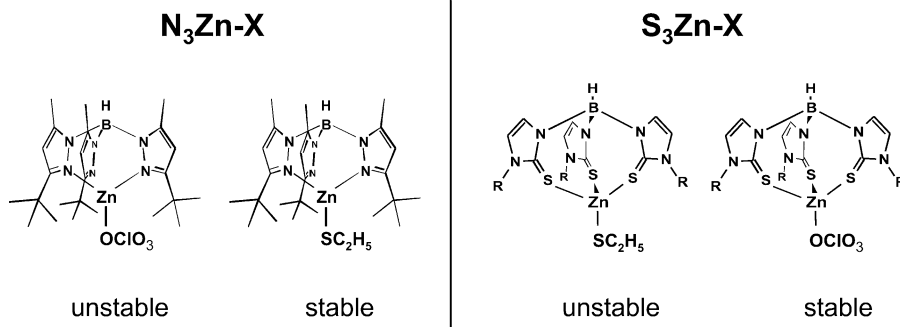
**Scheme 2** Hard–hard, soft–soft, and hard–soft ligand combinations in tripod zinc complexes.
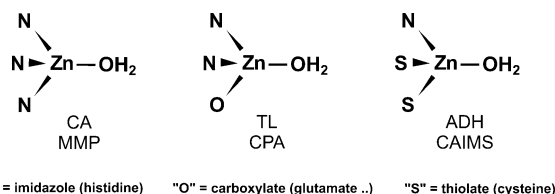
Scheme 3 Coordination motifs in the resting states of zinc enzymes. Abbreviations: CA carbonic anhydrase, MMP matrix metalloproteases, TL thermolysin, CPA carboxypeptidase A, ADH alcoholdehydrogenase, CAIMS cobalamin independent methionine synthase. As a result of the charge of the amino acid ligands the overall charge of the "zinc complexes" is +2 for the first group, +1 for the second and 0 for the third group.

of zinc and its environment in the enzyme must account for a pK_a value of the zinc-bound water molecule which is close to 7.

There can be no doubt that the low coordination number of zinc in its enzymes is the essential feature determining the low pK_a. The simple reasoning behind this is that the smaller the number of donors around a metal ion, the more electron density it will withdraw from each single donor, and for a water molecule as a donor this means increasing its acidity. There is quite a wealth of data on the acidity of hydrated zinc complexes.[17,19] They do not reveal a simple relation between coordination number and pK_a, as of course the coordination number is not the only influential variable. But the trend is evident, and the above-mentioned case of zinc chloride in water is a striking example among the simple zinc complexes.

During studies by coordination chemists aimed at the modelling of zinc enzymes, many polydentate ligands have been designed which leave room for just one water ligand in the coordination sphere of zinc.[20] In those cases where the acidities of these complexes have been determined they provide evidence for the coordination number–pK_a relationship. Thus, LZn(OH₂) complexes with L being a tetradentate ligand have pK_a values at or above 8.[21] But, most strikingly, all attempts to prepare LZn(OH₂) complexes with tripodal ligands (*i.e.* scorpionates in Trofimenko's terminology) have resulted in the isolation of their deprotonated derivatives, the LZn–OH complexes. We, among others,[20] have exploited this and prepared and studied the complexes listed in Scheme 4 as models for zinc enzymes.[22–24]
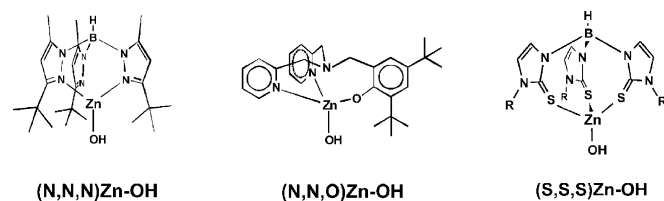

Scheme 4 Hydrolytically active Zn–OH complexes.

Much of our own work—and that of our friend and competitor Gerard Parkin[20]—has been done with pyrazolylborate zinc complexes as enzyme models.[25] Accordingly, these complexes have also provided a large amount of information on possible interactions between LZn species and the constituents of H₂O. The most difficult task, which is not fully achieved as yet, is the isolation of a Tp*Zn(OH₂) cation (Tp* denotes any one substituted tris(pyrazolyl)borate ligand), the main reason for which is the hydrolytic destruction of the Tp* ligands in acidic media.

Parkin came closest with the hydrogen-bonded salt Tp*Zn–OH–H · · · OH–B(C₆F₅)₃.[26] We encountered similar dinuclear complexes [Tp*Zn–OH · · · H · · · HO–ZnTp*]⁺ which formed from Zn(ClO₄)₂ in neutral solutions.[27] Their crystallization at neutral pH can be taken as evidence for the fact that the pK_a of Tp*Zn–OH is near 7 (*i.e.* an acid is 50% deprotonated when pH = pK). In all cases studied, the Tp*Zn–OH complexes are the easiest to prepare.[22,28,29] In just a few cases they could be induced to release water and form the dinuclear complexes [Tp*Zn–OH–ZnTp*]⁺,[24] and finally full deprotonation with formation of the neutral molecular species Tp*Zn–O–ZnTp* could be achieved in one case.[28] Thus, as summarized in Scheme 5, all constituents of water could be attached to zinc in all possible variations in mono- and dinuclear complexes.

$$Zn—OH_2 \qquad Zn—OH \qquad Zn—OH—Zn$$
$$Zn—OH\cdots H\cdots HO—Zn \qquad Zn—O—Zn$$
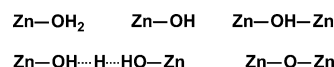
Scheme 5 Observed coordination types of pyrazolylborate zinc units with the constituents of water.

The Tp*Zn–OH complexes owe their stability both to the fact that the sterically laden Tp* ligands enforce a low coordination number and that they create a hydrophobic pocket around the Zn–OH unit. This is demonstrated in Scheme 6 by the phenyl- and cumenyl-substituted representatives which have pleased us with such a wealth of reactions that we have called them the Freiburg Enzyme Model. The space filling molecular model in Fig. 1 visualizes the encapsulation of the Zn–OH unit: on all sides but one it is concealed by the aromatic substituents; the open side, just as in the enzyme, is the access and removal path for substrates and products.
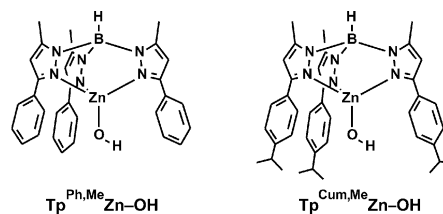
Scheme 6 The Freiburg Enzyme Model: the complexes Tp^{Ph,Me}Zn–OH and Tp^{Cum,Me}Zn–OH.

That access is possible and that potential substrates can occupy additional coordination sites at zinc, is demonstrated by the related structure in Fig. 2. Increasing the polarity (hardness) near zinc, in this case by a pyridyl substituted Tp* ligand, allows the coordination number to be increased, as mentioned above. As a result, the hydrolytic agent water and the organophosphate, employed to model the substrate, are attached to zinc at the same time.[30]

The Tp*Zn–OH complexes were shown to be able to perform, in a stoichiometric way, all hydrolytic reactions catalyzed by zinc enzymes, as outlined elsewhere.[20,25] Bearing voluminous organic substituents, they suffer from two limitations with respect to catalytic action. They are hydrophobic and do not dissolve in water, thereby rendering the reagent H₂O unavailable, and they form very stable complexes with the carboxylates and phosphates resulting from hydrolysis, *i.e.* they undergo product inhibition. On one hand this is unfavourable and still requires efforts in
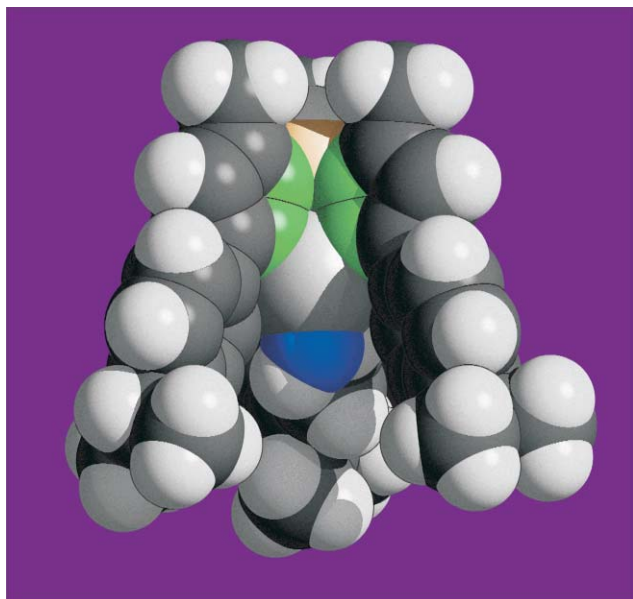
Fig. 1 Space filling representation of the structure of $Tp^{Cum,Me}Zn–OH$.

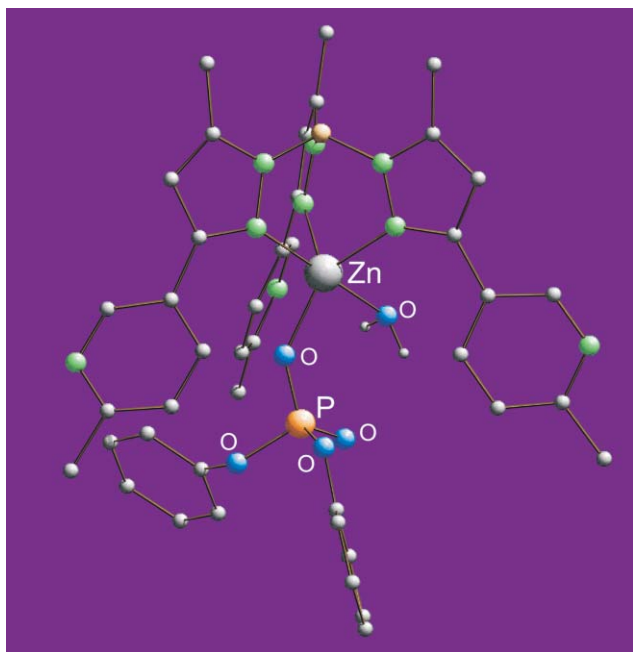**Fig. 2** Ball and stick representation of the enzyme-substrate model $Tp^{Py,Me}Zn(OH_2)OPO(OPh)_2$.

order to overcome it (see below). But on the other hand it has provided important compounds for the structure correlation based mechanistic findings (also see below).

Learning by failing

In the model complexes as well as in the enzymes the Zn–OH function represents the activated form of water, characterized by its easy accessibility (due to the pK_a of the Zn–OH$_2$ precursor) and high nucleophilic strength. This suggests that the related functions Zn–SH, Zn–OR and Zn–SR should have similar properties, either in terms of acid–base behaviour or in terms of reactivity. Our investigations of Tp*Zn complexes with these functional ligands revealed some similarities, but it was the dissimilarities that increased our understanding of the Zn–OH function.

Tp*Zn–SH complexes

The Tp*Zn–SH complexes are remarkably easy to prepare: from Tp*Zn–OH with H$_2$S[31] or in a quite unusual reaction with CS$_2$.[32] Considering the generally high lability of metal hydrosulfide complexes, they are rather stable and, as it turned out, of low reactivity. Although there is no reason to assume that the nucleophilicity of Tp*Zn–SH is lower than that of Tp*Zn–OH, this is not borne out by thiolytic cleavage of hydrolyzeable substrates. Neither esters, amides, organophosphates nor CO$_2$ show any reaction with Tp*Zn–SH. The message learnt from this was that the SH function lacks the lability, *i.e.* leaving group quality, of the OH function, due to the high affinity between zinc and sulfur.[32]

On the other hand, protonation or alkylation, *i.e.* converting the SH ligand to a H$_2$S or RSH ligand, resulted in high lability. Acids HX convert the Tp*Zn–SH complexes to H$_2$S and Tp*Zn–X, and methyl iodide produces CH$_3$SH and Tp*Zn–I.[31] This observation points to an essential difference between the Zn–OX and Zn–SX functionalities: the former has to be produced from HOX (*e.g.* H$_2$O) to be labile and hence suitable for a catalytic cycle, while the latter has to be converted to a HSX or RSX species for the same purpose.

Tp*Zn–OR complexes

No other class of Tp*Zn–X complexes is less pleasant to work with than the alkoxides. The smallest trace of water will hydrolyze them to their hydroxides, which means that they have no reliable existence outside the glove box.[33–35] In addition to water every acid HX, even such weak ones as amides or acetonitrile, will release the alcohol and form the corresponding Tp*Zn–X. Thus the lability of the Zn–OH function is more than amply reproduced by the Zn–OR function.

The difference between Zn–OH and Zn–OR shows up in the solvolytic reactions. Except for the most reactive species, esters or organophosphates are not cleaved (*i.e.* transesterified) by Tp*Zn–OR. The notable exception to this rule is provided by thioesters which are converted to esters and Tp*Zn–SR. The general difference between the Zn–OH and Zn–OR reactivities, *i.e.* hydrolysis *vs.* transesterification, is of course explained by the lack of a mobile proton in the Zn–OR complexes. But the ease of reaction for the thioesters also shows that the strength of the resulting zinc–substrate bonds has a major influence upon the occurrence of these reactions. This also has mechanistic implications which will be discussed below.

Tp*Zn–SR complexes

The Tp*Zn thiolates are the most convenient in this group of complexes, as they are air and water stable and easy to make. Not suffering from the lability of the Tp*Zn–OH and Tp*Zn–OR species, they are suitable in order to show the differences between the Zn–OX and Zn–SX functionalities. As already mentioned for the Tp*Zn–SH complexes, the major feature of their reactivity is their lability after electrophilic attack. This may be a laboratory curiosity for the reaction of Tp*Zn–SH with methyl iodide, but it

is the essence of catalysis in a unique class of zinc enzymes, the thiolate alkylating enzymes.[14]

In these enzymes the thiol, for example homocysteine, is first attached to zinc as a thiolate and then subjected to the attack of the alkylating agent. All the steps of the catalytic cycle can be reproduced nicely with the Tp*Zn·SR complexes, see Scheme 7.[14] The most significant step is the fast release of the resulting thioether, in accord with the fact that until today no zinc complex of a simple thioether, mercaptan or of H_2S has been isolated, in contrast to the multitude of H_2O or ROH complexes. Zinc is unique in this respect, as compared to copper or iron, another non-property that makes it useful in Nature.
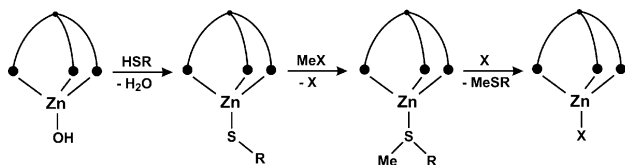

Scheme 7 Stepwise reaction sequence for the alkylation of a thiol with Tp*Zn–OH.

Mechanism from structure

The biomimetic chemistry performed with pyrazolylborate zinc complexes has the disadvantage that almost all reactions are stoichiometric rather than catalytic. In terms of coordination chemistry and an understanding of mechanisms, however, this is an advantage: the isolated species can represent successive steps of a catalytic sequence, in favourable cases even transient intermediates on the slope of a reaction coordinate. Two examples for this out of our own research are presented here.

Zinc and CO_2

Everywhere in living organisms carbon dioxide is produced or consumed. The zinc enzyme carbonic anhydrase,[36] which catalyzes the hydrolytic interconversions between CO_2 and bicarbonate, is essential for many of these processes. The reactive form of this enzyme is again the Zn–OH species. Many experimental and theoretical studies[36,37] have resulted in the proposed reaction pathway given in Scheme 8. The important features of this pathway are the initial four-center intermediate, its evolution by making and breaking of one Zn–O bond each, and the positioning of the bicarbonate proton. In the case of the latter the two alternatives, namely the Lindskog or Lipscomb pathways, are still being discussed.
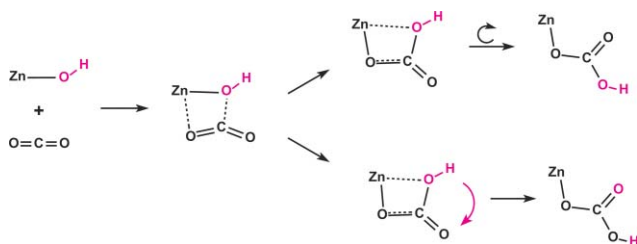
Scheme 8 Proposed pathways for the activation of CO_2 by carbonic anhydrase. Upper sequence: Lindskog mechanism. Lower sequence: Lipscomb mechanism.

We tried to model carbonic anhydrase early on with our Tp*Zn–OH complexes.[22] But neither we nor our competitors have been able to isolate a zinc-bicarbonate complex. Yet, the insertion of CO_2 into other Zn–X functions and the use of heterocumulenes instead of CO_2 have provided us with enough compounds and their structures to allow us to visualize a model of the L·Zn–OH/CO_2 reaction course.[33,38] The main results of these studies with Tp*Zn–X species are the following:

1. All insertion products retain a "memory" of the four-center mechanism in their structures. The bicarbonate analogues resulting from the heterocumulene insertions are always bound to zinc in a bidentate fashion, sometimes very clearly as in Fig. 3, sometimes rather weakly. The superposition of all structures produces a nice image of the second step in Scheme 8, the breaking of the Zn–OH bond and the formation of the new Zn–O bond.
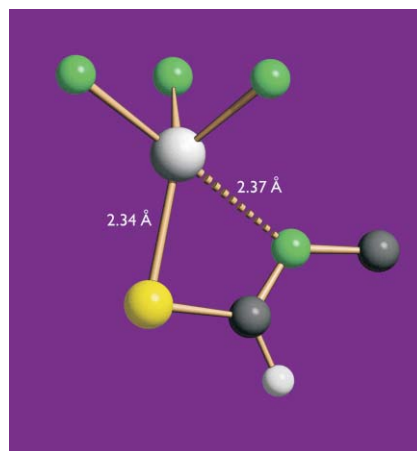

Fig. 3 Central part of the structure of $Tp^{Ph,Me}Zn–S–CH=NC_6H_4NO_2$, resulting from the insertion of RNCS into $Tp^{Ph,Me}Zn–H$.

2. Evidence for the four-center mechanism can also be derived from alternative product constitutions, as shown in Scheme 9. The most basic terminus of the heterocumulene is approached by the zinc ion and kept there, resulting in two different modes of insertion for isocyanate and isothiocyanate into the Zn–OMe function.
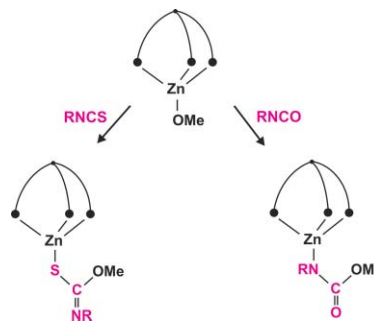
Scheme 9 Alternative orientations of heterocumulenes upon insertion into Tp*Zn–OMe.

3. Both the Lindskog mechanism (rotation of the intact COOH unit) and the Lipscomb mechanism (transfer of the proton from one O atom to the other) can be reproduced by our structures. Fig. 4 displays the alternative orientations of the COOMe ligands
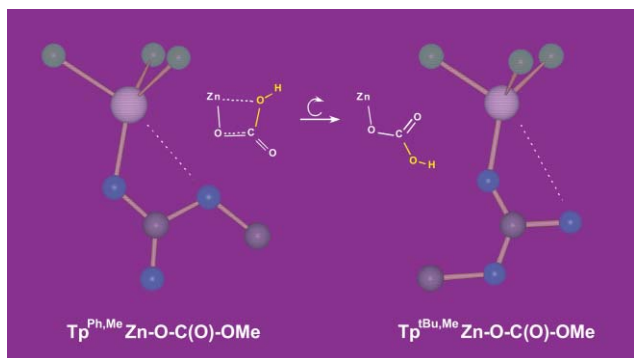
Fig. 4 Central parts of the structures of two Tp*Zn–OCOOMe complexes as models for the Lindskog interconversion (inset).

in two different Tp*Zn–OCOOMe complexes. Taking OMe as a substitute for OH, the "rotation" from the first structure to the second is an image of the Lindskog process.

In contrast, Fig. 5 shows images for the alternative positions of the mobile proton in the Lipscomb process. On the left the initial state is frozen by substituting OMe for OH, *i.e.* not allowing a migration. On the right the mobile proton has migrated from the original Zn–OH situation to the thiocyanate's N atom.
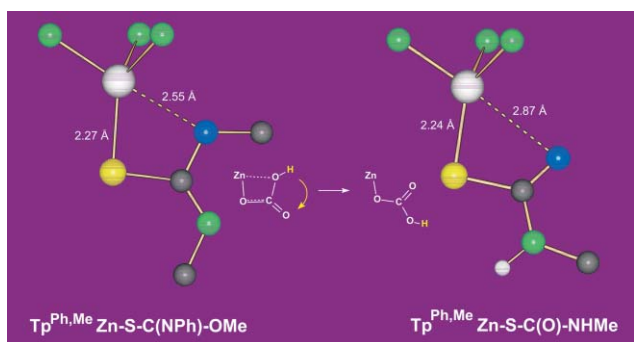

Fig. 5 Central parts of the structures of Tp*Zn complexes resulting from the insertion of isothiocyanates into the Zn–OMe (left) and Zn–OH functions (right) as models for the Lipscomb interconversion (inset).

All observations made here are also compatible with the continuation of Scheme 8 as given in Scheme 10. Either proton migration or COOH rotation can reconvert the bicarbonate complex to a four-center intermediate which is not identical to the initial one. From this intermediate CO_2 can be released again. However, when the oxygen of the original Zn–OH complex had been labeled, the label could now be carried by the CO_2. This is the reason why labeling studies are of no help when studying carbonic anhydrase or even its model complexes.[39]

Structure correlation analysis

The hydration of CO_2 by carbonic anhydrase is one specific case of the ubiquitous hydrolytic activities of zinc enzymes. In all other cases the essential Zn–OH function not only has to attack the electrophilic center of a substrate, but also has to assist in the removal of the leaving group during the hydrolytic cleavage. This means for example that the initial four-center intermediate, depicted below in analogy to the Zn–OH/CO_2 situation, must
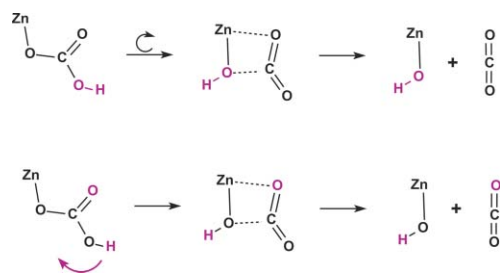
Scheme 10 Continuation of Scheme 8 for the release of CO_2, showing that the colour-coded oxygen which came from the initial Zn–OH function can be incorporated into the CO_2 molecule.

evolve along a low-energy pathway to a Zn–OC(O)R complex and free HX.
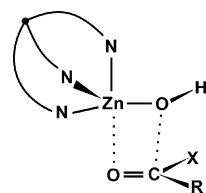
The inhibition of zinc enzymes by hydroxamates[40] has given us the clues as to what this low-energy pathway may look like. The reasoning behind this is the proposal that the structures of enzyme-inhibitor complexes are transition state analogues,[41] *i.e.* they represent "frozen" situations near the transition state of the catalytic process. Typical inhibitors like the hydroxamates, however, are bidentate. The transition state analogy then means that near the transition state of the enzymatic process both oxygen atoms (that of the OH function and that of the substrate) are still bound to zinc, as can quite naturally evolve from the encounter complex depicted above.

The further notion that simple zinc complexes of hydroxamates can model the enzymatic transition state analogues became realistic by our finding that the primary coordination sphere of zinc in the $Tp^{Cum,Me}$Zn–acetohydroxamate is superimposable onto that in a hydroxamate-inhibited Class II aldolase zinc enzyme.[42] Furthermore the geometries at the five-coordinate zinc, in the hydroxamate-inhibited enzymes as well as in the Tp*Zn-hydroxamate complexes, can vary considerably, never being purely trigonal bipyramidal or square pyramidal.[40,42]

With this background it seemed appropriate to us that the system in question is suitable for the method of structure correlation analysis, developed and used so successfully by Bürgi and Dunitz.[43] This method requires that a sufficiently large number of structures is known for a group of compounds which bear a relation to a reaction mechanism. Superimposing the essential parts of all these structures then reveals "static" and "mobile" sites in the molecules. In the successful cases of structure correlation analysis the "motion" of the mobile sites defines the trajectory of the bond-breaking or bond-making chemical reaction.

In our case we had a large body of structural information for Tp*Zn complexes. Out of these, the species Tp*Zn(X)(Y) with five-coordinate zinc, either with two monodentate ligands X and Y or with one chelating ligand XY, were the ones to be studied. At the time of our study[12] there were 30 complexes in this group. The superposition of their structures yielded the desired result: there

is comparatively little "motion" on the side of the Tp* ligand (the backbone of the enzyme), but the positions of the X and Y ligands span almost the full range of geometries between trigonal bipyramidal and square pyramidal. Fig. 6 shows a simplified version of this structure correlation, limiting the number of mobile atoms X and Y to 8 each.
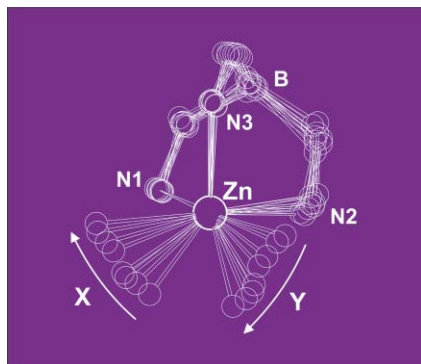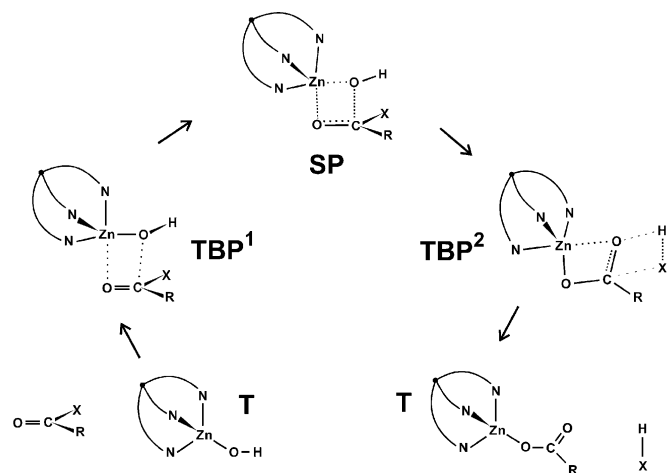

Fig. 6 Structure correlation by the superposition of the immediate zinc coordinations of eight Tp*Zn(X)(Y) complexes.

Translated into a reaction mechanism, the ligand Y in Fig. 6 represents the incoming group and ligand X the leaving group. For the hydrolysis of an ester or amide, X is the Zn–OH oxygen and Y is the carbonyl oxygen of the substrate. It is clearly visible that Y enters on the axis of a trigonal bipyramid and finally occupies a tetrahedral position. Conversely, X, which originally occupied the tetrahedral position finally leaves along the axis of a different trigonal bipyramid. The whole process follows the symmetry laws of the Berry pseudorotation, and halfway through, when the Zn–X and Zn–Y interactions are equally strong, the symmetry at zinc is square pyramidal.

In Scheme 11 the mechanistic information is expressed in molecular formulae. If one would start from the square pyramidal transition state in the center, the sequences to the left and to the right are symmetry-equivalent, but the bonds forming and breaking are different. The oxygen bound to zinc in the product formerly was the carbonyl oxygen of the substrate, and the initial

Zn–OH oxygen becomes the carbonyl oxygen in the product. This is the essence of the four-center mechanism in zinc enzyme catalysis, and the two non-properties of zinc, the non-preference for any coordination geometry and the non-inertness of Zn–O bonds, make it possible.

Last but not least: catalytic Tp*Zn chemistry

As mentioned before and as seen above, the Tp*Zn complexes under investigation did not lend themselves to catalytic reactions. Their organic substituents disfavor access of water to the functional Zn–X center, but favor product inhibition, *i.e.* the formation of uncharged molecular complexes by attachment of the hydrolysis products to zinc. The way out of this dilemma required a more hydrophilic Tp*Zn chemistry by the incorporation of suitable substituents in the Tp* ligands. This is not a trivial task, as these ligands are hydrolyzeable boron–nitrogen compounds and their standard synthesis requires temperatures up to 200 °C.

Our pyridyl-substituted Tp* ligands were a first step in the desired direction.[44,45] Following a recent approach by Trofimenko,[46] we also became able to attach carboxamido substituents[45] and thereby finally obtain a few water-soluble Tp*Zn–X complexes. The price for this progress was the loss of tetrahedral coordination. In the solid state all the new complexes are five-coordinate, as a rule by coordination of donor atoms on the polar Tp* substituents. The Tp* ligands that were best suited for our purposes were the following:
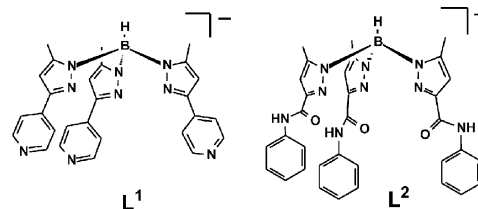
In solution ligands **L¹** and **L²** form zinc-aqua complexes which are stable in DMSO–water or methanol–water solutions containing the MOPS buffer.[47] In these solutions they catalyze the hydrolysis of *p*-nitrophenyl acetate and tris(*p*-nitrophenyl)phosphate (TNP). The best catalyst is that with **L²** which is able to remove two *p*-nitrophenyl groups from TNP. The catalytic activities are not yet exciting, with turnover numbers around 4, but they marked a very satisfying conclusion of our work in this field.

Another catalytic reaction came as a surprise: complex Tp^{Ph,Me}Zn–OH was found to catalyze the methanolysis of β-butyrolactone and *N*-*p*-nitrophenyl substituted β-lactams,[48] as exemplified here for the lactone:
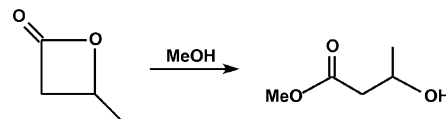
It could be made plausible that the catalytically active species is not the Zn–OH complex, but the powerful nucleophile Tp^{Ph,Me}Zn–OMe which exists in minute concentrations in Tp^{Ph,Me}Zn–OH– methanol solutions as an equilibrium species. The catalytic efficiency is humble again, and the value of these findings again lies in the fact that our workhorses, the Tp*Zn–OH complexes, can induce catalysis after all.

Scheme 11 Mechanistic pathway, as derived from structure correlation analysis, for the cleavage of an ester or peptide by Tp*Zn–OH, Abbreviations: T tetrahedral, TBP trigonal bipyramidal, SP square pyramidal.

These findings marked the end of my scientific career. If I were a young researcher, they would mark a beginning. Zinc in its non-spectacular corner of the periodic table, which once I called the boring element, has not been boring at all for us, and I can only wish that our successors will have as much fun with it as we did.

Acknowledgements

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