DRUG DESIGN OF ZINC-ENZYME INHIBITORS
FUNCTIONAL, STRUCTURAL, AND DISEASE APPLICATIONS

Edited by
Claudiu T. Supuran
Jean-Yves Winum
DRUG DESIGN OF ZINC-ENZYME INHIBITORS
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In recent years, the life sciences research has considerably attracted scientists to investigate metalloenzymes and their modulators of activity (inhibitors and/or activators) to meet the challenges for improving human health by discovering new therapeutic targets.

This book mainly deals with the progress that has been made in the field of drug design and discovery of zinc metalloprotein inhibitors over the past years. Recent trends in zinc metalloenzymes are structured into five parts, comprising 40 chapters contributed by experts in the field from all over the world. Of these contributors, there are many who have contributed to this area for decades as scientists and have been recognized for the same.

The five parts of this book can be read as a whole or individually, independent of each other. In fact, the book not only caters to academic or industrial researchers in any of the areas related to pharmaceutical research and development but also to advanced undergraduates as well as graduates at the beginning of their research career, interested in specific topics of this field.

Part I (Chapter 1) outlines the importance of the zinc ion in biological systems and focuses on the importance of targeting zinc enzymes as a promising strategy for drug design and development.

Part II (Chapters 2–22) deals with one of the most studied zinc enzymes among all metalloproteins, the carbonic anhydrase (CA), and provides a comprehensive up-to-date review on the development of modulators of activity for both eukaryotic and prokaryotic CAs and their potential use in drug discovery. Part III (Chapters 23–27) brings to light the potential of matrix metalloproteinase/ADAM inhibitors as drug candidates. Part IV (Chapters 28–31) discusses the relevance of bacterial zinc protease as potential drug target and the use of inhibitors as anti-infective agents. Finally, Part V (Chapters 32–40) reviews the current and potential clinical applications of other zinc-containing enzymes in the treatment of cancer and viral and bacterial infections.

All the data given in this book provide a chemical, biological, and pharmacological framework for understanding the clinical utility of compounds targeting zinc metalloproteins for the treatment of various diseases.


We express our deepest gratitude to all our coworkers and colleagues who have contributed their highly informative manuscripts to this book on time and without whom this book would not have been possible.
We would also like to thank Prof. Binghe Wang who got this book included in the important collection of Wiley book series on drug discovery and development, which he is editing.

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PART I

INTRODUCTION
1.1 INTRODUCTION

Drug target is an old concept that was suggested at the end of the nineteenth and the beginning of the twentieth century by Ehrlich and Langley who developed the idea that compounds display biological activity by binding to cellular constituents.\(^1\)

Commonly, most of the drug targets can be defined as human genome-derived proteins (or proteins belonging to pathogenic organisms) that undergo a selective interaction with compounds administered to treat or diagnose a disease.\(^2\) Target identification and validation constitute the most important steps in the process of drug discovery. At present, there is an enormous interest in identifying and validating "druggable" targets in the human proteome and applying structure-based drug design to discover new therapies for important human diseases. The human genome is a huge reservoir of putative drug targets, and its sequencing has allowed identification of about 8000 targets of pharmacological interest. Nevertheless, for all classes of approved therapeutic drugs, around 300 targets have been disclosed with increasing frequency: 270 being encoded by the human genome and the remaining belonging to pathogenic organisms.\(^3,4\) Several promising targets for drug intervention have been revealed in recent years, and their knowledge is helpful for molecular dissection of the mechanism of action of drugs and for predicting features that guide new drug design and the search for new targets.
According to Imming et al., drug targets can be divided into several categories: (i) enzymes, (ii) substrates, metabolites, and proteins, (iii) receptors, (iv) ion channels, (v) transport proteins, (vi) DNA/RNA and the ribosome, (vii) targets for monoclonal antibodies, (viii) various physicochemical mechanisms, and (ix) unknown mechanisms of action.

Among these different classes of drug targets, enzymes have long been considered valuable drug targets for the treatment of major human diseases, as several thousands of enzymes are encoded in the human genome, and they play a key role in virtually every physiological/pathological process. At present, at least 66 human enzymes and 20 bacterial, viral, fungal, or parasite enzymes are targets of approved drugs, for example, up to 40% of the known drug targets. Enzymes containing metals (metalloenzymes) are of increasing interest and importance, as the genetic consequences of metalloprotein regulation become better understood. The largest category of metalloproteins is constituted by zinc enzymes, with more than 300 representatives presently known, covering all major six enzyme classes established by the International Union of Biochemistry (i.e., oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases). Over the past few years, substantial evidence has been accumulated implicating the zinc enzymes in the pathophysiology and pathogenesis of a variety of human disorders ranging from infections to cancer. The relevance of zinc metalloproteins to biomedical sciences has increased much in the past few years, and modulation of their activity with small-molecule drugs, designed to interact with a clearly defined ligand binding site, constitutes a challenging area in drug design and discovery. Furthermore, the availability of different high-resolution X-ray crystal structures of such enzymes and of their complexes with substrates and/or inhibitors has provided a wealth of information with a profound effect on the way we understand their biological functions.

In this chapter, we present an overview of the role of zinc in biological systems and explain why zinc proteins constitute promising targets for drug intervention.

1.2 IMPORTANCE OF ZINC IN BIOLOGICAL SYSTEMS: STRUCTURAL, REGULATORY, AND CATALYTIC ROLES

Among the transition and group II elements, zinc is the second most abundant metal, after iron, in all biological systems including microorganisms, plants, and animals. It is stable as dication (Zn$^{2+}$), has Lewis acid properties (it can accept a pair of electrons), and lacks redox activity, as it possesses a full d-shell d$^{10}$ orbital. This ubiquitous element is considered an essential, nontoxic micronutrient, and its several biochemical roles regarding the structure and function of proteins, including enzymes, transcription factors, hormonal receptor sites, and biological membranes, have been recognized. Zn(II) is highly regulated under normal physiological conditions, as this metal ion plays a key role in a wide variety of processes such as DNA and RNA synthesis, transmission of the genetic message, growth and development, signal transduction, apoptosis, brain and immune function, lipid metabolism, and so on.
In addition, the zinc ion is also closely involved in intracellular signaling and neuromodulation functions.\textsuperscript{7,8}

Physiologically, approximately 98% of the total zinc in an organism is found within the cell (40% in the nucleus and 50% in cytoplasm, organelles, and specialized vesicles), while the remaining is found in the cell membrane.\textsuperscript{7,9} The total zinc concentration in eukaryotic cells was reported to be in the high micromolar range, with a concentration around 200 \(\mu\text{M}.\textsuperscript{10}\) Furthermore, zinc deficiency is detrimental in many aspects to the normal function of the organism, with notable effects on growth and immune functions.\textsuperscript{7} The cytosolic concentration of free \(\text{Zn}^{2+}\) is very low and can be estimated in the subfemtomolar range, but it increases under oxidative stress conditions.\textsuperscript{11}

At the molecular level, the intracellular \(\text{Zn}^{2+}\) is most often tightly bound to proteins considered an essential cofactor for hundreds of enzymes and thousands of metabolic and regulatory proteins, fulfilling both structural and catalytic roles.

1.2.1 The Structural Role of Zinc

Zinc plays an important role in the structure of proteins and cell membranes. In such structural site, it can be found either as a single metal ion or as part of a cluster of two or more ions, being coordinated only by amino acid residues with no bound solvent molecule(s). Thus, the metal ion ensures an essential role in the stabilization of the protein structure by creating or maintaining secondary/tertiary structural elements in the same manner as a disulfide bridge.\textsuperscript{12} It can induce the correct folding of protein sequences as zinc fingers, zinc twists, or zinc clusters in numerous regulatory proteins and hormone receptors, contributing to the overall stability of these domains.\textsuperscript{13} Zinc fingers are structurally diverse and are present in proteins that perform a broad range of functions in various cellular processes. The biological functions of zinc-containing proteins strongly depend on the zinc ion, which ensures integrity and stability and is critical for binding to DNA.\textsuperscript{13} These structure-stabilizing motifs are as diverse as their functions and are associated with protein–nucleic acid recognition as well as protein–protein interactions.\textsuperscript{14,15} The zinc ion may also be involved in the maintenance of the structure of chromatin and biomembranes, as it plays a crucial role in the regulation of their functions.\textsuperscript{14}

The biological function of zinc is governed by the composition of its flexible coordination sphere. This can be a slightly distorted tetrahedral or a trigonal bipyramidal coordination polyhedron in most metalloproteins, with the metal ion coordinating three or four amino acid residues.\textsuperscript{8,16–19} Structural sites are typically characterized by a zinc-centered tetrahedral coordination in which the metal ion is fully coordinated by four Cys residues via thiolate group, generally separated from a relatively short sequence in the protein (Fig. 1.1a). Other ligands may also compete with cysteines for binding \(\text{Zn(II)}\); the second most prevalent ligand is His, which is usually found in combination with Cys, forming structurally related “zinc finger” motifs (Fig. 1.1b).\textsuperscript{16–19} Examples of non-Cys structural zinc site have also been reported, apart from the catalytic zinc site in matrix metalloproteinase (MMP) class

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of enzymes, with combinations of His and Asp residues coordinating the metal ion (Fig. 1.1c).

Structural zinc sites have important implications for the functioning of metalloproteins. By stabilizing and even inducing the local folding of protein subdomains in the immediate neighborhood of the metal site, one or more amino acid side chains can be orientated toward the active site, thus influencing the enzyme activity by affecting the chemical environment of the active center and/or by influencing the alignment of active site residues for catalysis. 16,17

Besides structural zinc sites involved in protein functions, a number of protein interface zinc sites can be defined, where the zinc ion bridges proteins or their subunits, thus playing an important role in the organization of the quaternary structure and/or active site of the protein. 16,17,20,21 In these structural sites, zinc ions bridge the interfaces of proteins via ligands provided by different polypeptide chains and can cross-link the same protein leading to homodimers/trimers or tetramers. The link of two different proteins through such intermolecular ligands has also been observed. In such cases, zinc ligation is assumed by coordinating not only residues such as His, Glu, and Asp but also Cys, with two amino acid ligands supplied by both protein moieties or three amino acid ligands coming from one protein backbone and one ligand from another protein domain. The resulting protein interface zinc binding sites can function as catalytic, cocatalytic, or structural sites, playing a key role in transduction pathways that regulate a host of cellular functions. 16–21

1.2.2 Catalytic and Cocatalytic Role of Zinc

In catalytic sites, zinc ions participate directly in the catalytic process and generally exhibit a distorted tetrahedral geometry with only three O/N/S ligands bound to the zinc ion, the fourth ligand being a water molecule that is an activated nucleophile for the catalytic process. The coordination number 5 can also be encountered for Zn(II) ions, with a trigonal bipyramidal geometry of the metal center. The zinc ion is essential
for catalytic activity of more than 300 enzymes and is located at the core of the enzyme active site, participating directly in the catalytic mechanism through interaction with substrate molecules undergoing the transformation. The most common coordination feature of Zn(II) in catalytic sites is dominated by histidine side chains coordinating the metal ion, through interaction with the Né atom of the imidazole ring. Other coordinated amino acid residues are Glu, Asp, and Cys. Due to the amphoteric properties of Zn$^{2+}$, a water molecule always participates in the coordination sphere as a fourth or fifth ligand. Catalytic zinc binding sites of some representative metalloenzymes are illustrated in Fig. 1.2, where the ligands (L) are three His residues for the lyases carbonic anhydrases (CAs) and two His residues for the hydrolase carboxypeptidase. The catalytic zinc site of alcohol dehydrogenase is the only one known so far where there is only one His residue bound to the metal ion, being also unique as two cysteine residues participate in the coordination.

This zinc-bound water molecule is crucial for the catalysis promoted by the metal center, as it can be either involved in the catalytic process as hydroxide ion or activated via polarizing effect of the neighboring amino acids of the active site, acting as a nucleophile per se.$^{16,17}$ Moreover, H$_2$O can be displaced by the substrate (S) or expanded upon interaction with the substrate (Fig. 1.3). The presence of water molecules in the coordination sphere is usually a distinguishing feature that allows to differentiate a catalytic zinc site from a structural one.$^{12}$
Cocatalytic zinc sites can also be distinguished and are characteristic of multi-zinc-containing enzymes, with two or three metal ions in proximity, with two of them bridged by a side chain moiety of a single amino acid residue, such as Asp, Glu, or His, and sometimes a water molecule. Asp and His are preferred amino acids for these sites. No Cys ligands are found in such sites. Typically, the metal ions are separated by a short distance (around 3 Å) and bridged by at least one common ligand, frequently a water molecule or a carboxylate ligand of those mentioned above. The zinc ion can be bridged with another zinc ion or with another metal ion, such as Cu(II), for example, in Cu–Zn superoxide dismutases (SOD).  

In the past decade, there has been a great expansion in our knowledge of the role of metalloenzymes in the physiopathology of several diseases. Catalytic zinc sites provide convenient targets for drug intervention and the design and development of small-molecule drugs that can coordinate directly to the metal, displacing the zinc water in the active site and inhibiting the enzymes. This challenging research area has been extensively dealt with in this book.

### 1.3 Zinc Metalloproteins as Drug Targets

#### 1.3.1 Targeting Human Zinc Metalloenzymes

Since the identification of the first metalloenzyme, carbonic anhydrase, by Keilin and Mann in 1941, more than 300 different enzymes requiring zinc as essential cofactor have been identified, showing their diverse and important physiological functions.
These enzymes are considered to be very attractive targets for drug therapy, and their inhibitors are included in the armamentarium of modern medicine against human diseases such as cardiovascular, neurological, infectious, and metabolic diseases, as well as cancer.\(^5,6\)

Considering the importance and the diversity of zinc-containing enzymes, this book will focus on the zinc enzymes that are relevant for biomedical applications due to their well-known role in life-threatening diseases. For example, the two most investigated metalloproteins that will be considered in detail in this book are the carbonic anhydrases (dealt with in Part II) and the matrix metalloproteinases (dealt with in Part III).

Carbonic anhydrases (EC 4.2.1.1) that belong to the lyase family are ubiquitous zinc enzymes present in prokaryotes and eukaryotes, all over the phylogenetic tree. These are efficient catalysts for the hydration of carbon dioxide to bicarbonate and protons, playing crucial physiological/pathological roles in acid–base homeostasis, secretion of electrolytes, transport of ions, biosynthetic reactions, and tumorigenesis. These enzymes are of clinical relevance as some isoforms among the 15 known in humans are established drug targets, with many inhibitors that have been reported and developed as diuretics, antiglaucoma, anticancer, and antiobesity agents, or for the management of a variety of neurological disorders, including epilepsy and altitude disease.\(^23,24\) Furthermore, a clear connection has recently been found between CA inhibition and lipogenesis (thus, CA inhibitors might be used as antiobesity agents) as well as tumorigenesis (antitumor drugs/diagnostic tools).\(^23,24\) Thus, Chapters 2–22 will be dedicated not only to this class of enzymes and their inhibitors/activators from mammals (\textit{Homo sapiens} being the most investigated one) but also to the various CAs recently characterized from many bacteria, archaea, protozoa, fungi, yeasts, and nematode species. Many such enzymes are now fully characterized kinetically, and their inhibition/activation studies on many classes of compounds reported, thus constituting an important starting point for the rational drug design of inhibitors with clinical applications.\(^23,24\) Such research is very dynamic nowadays, and the near future may see the emergence of novel therapeutic agents targeting such enzymes.

Another essential class of zinc metalloproteins that will be taken into consideration is the superfamily of zinc endopeptidases, MMPs and ADAMs (a disintegrin and matrix metalloproteinase domain), which are dealt with in Chapters 23–27. MMPs are zinc endopeptidases that degrade both matrix and nonmatrix proteins. At least 23 MMPs are known in humans where they play an important role in morphogenesis and in a wide range of processes including tissue repair and remodeling. Their abnormal expression contributes to pathological processes, including arthritis, cancer, and cardiac and central nervous system diseases, and inhibition of MMPs has widely been sought as a strategy in the intervention of these disease processes.\(^5,6,25,26\) A large number of MMP/ADAM inhibitors showing selectivity for the various members of this superfamily have been reported in the past few years holding considerable promises mainly in the anticancer and cardiovascular therapy.

Other zinc metalloenzymes of medical relevance, such as angiotensin-converting enzyme (ACE), histone deacetylase, prostate-specific membrane antigen (PSMA), and protein farnesyltransferase, among others, have already demonstrated a crucial
therapeutic potential in various pathological, especially in cancer, neurodegenerative, and inflammatory diseases, and they are reviewed in Chapters 32–36. A special mention should be made of HIV integrase, which is a metalloenzyme containing zinc, (Chapter 37), but Zn(II) is not involved in the catalytic cycle (instead, it seems that Mg(II) is present at the active site). Considering the great importance that the treatment of HIV infection has nowadays gained and the fact that HIV integrase inhibitors were approved for clinical use in 2008, after a successful saga of research and development of more than 15 years, we decided to dedicate a chapter to this interesting zinc enzyme that is in fact not a real zinc enzyme. This is an exception, since, as the title mentions, the main focus of this book is the inhibition of zinc enzymes in which Zn(II) clearly has a catalytic role. Another very recent and quite promising antiviral target is constituted by the APOBEC3F/G family of enzymes that will be dealt with in Chapter 40.

### 1.3.2 Targeting Bacterial Zinc Metalloenzymes

Infectious diseases still remain the main cause of human deaths worldwide. The emergence and spread of pathogenic bacterial strains resistant to most classes of clinically used antibiotics have created the need for the development of such novel therapeutic agents as possessing a different mechanism of action. Development of new anti-infective agents with a novel mode of action and lacking cross-resistance to the existing drugs is a strong imperative of biomedical research of early twenty-first century, and a highly unaccomplished task until now. In the past 10 years, bacterial genome analysis allowed to define new essential bacterial genes and provided many details concerning the structure of bacterial proteins that play an important role in pathogenesis, with many such prokaryotic zinc metalloenzymes being identified. Metalloproteins that are essential for bacterial growth and are not required by mammalian cells constitute potential targets for antimicrobial drugs and form the basis for future therapies. Several of these orphan (for the moment) targets, such as bacterial proteases, botulinum, tetanus and anthrax lethal factors (LAs), clostridial collagenases, and other bacterial proteases, will be dealt with in Chapters 28–31.

Characterization of many specific as well as ubiquitous proteases in both Gram-positive as well as Gram-negative pathogens has allowed the development of different classes of specific nanomolar-range inhibitors for bacterial proteases such as *Clostridium histolyticum* collagenase, *Botulinum* neurotoxin, and *Tetanus* neurotoxin. Moreover, a number of approaches have been taken to identify inhibitors of the zinc-dependent metalloproteinase lethal factor, a critical component of anthrax toxin and an important potential target for small-molecule drugs.

Identification of zinc metalloenzymes from bacterial genomes has allowed identification of new potential targets for the development of anti-infective agents. This strategy, which has already demonstrated promising results, constitutes a challenging area, considering all the possible targets available in the zinc metalloprotein family with potential therapeutic applications.

Several other chapters of the book deal with zinc enzymes that are just beginning to be investigated in more detail, such as P-III metalloprotease from a highly poisonous
snake (Chapter 33), the histidinol dehydrogenases (which may constitute an interesting class of antibacterials, Chapter 38), or the dihydroorotase inhibitors (with potential for developing antimalarials, Chapter 39).

It is thus clear that the wealth of genomic, structural, biochemical, and synthetic data that has recently emerged in biomedical research of zinc enzymes and their inhibition enables us to dedicate this book to these fascinating fields. Although we clearly understand that due to the vastness of the field, it is not possible to deal with all important enzymes here, we have tried to make a comprehensive review of the literature data for the most relevant representatives, for their inhibitors, and for their biomedical applications.

REFERENCES


