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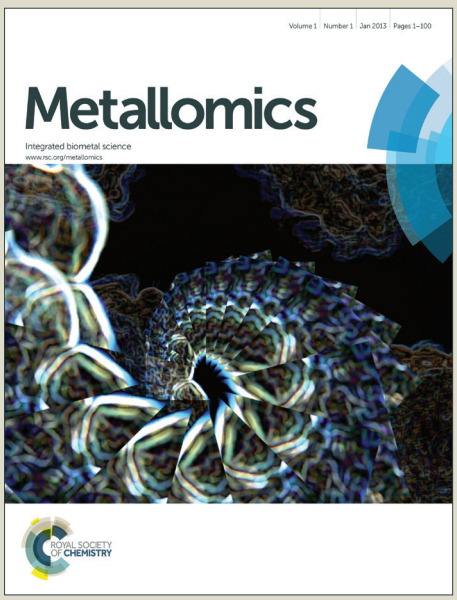

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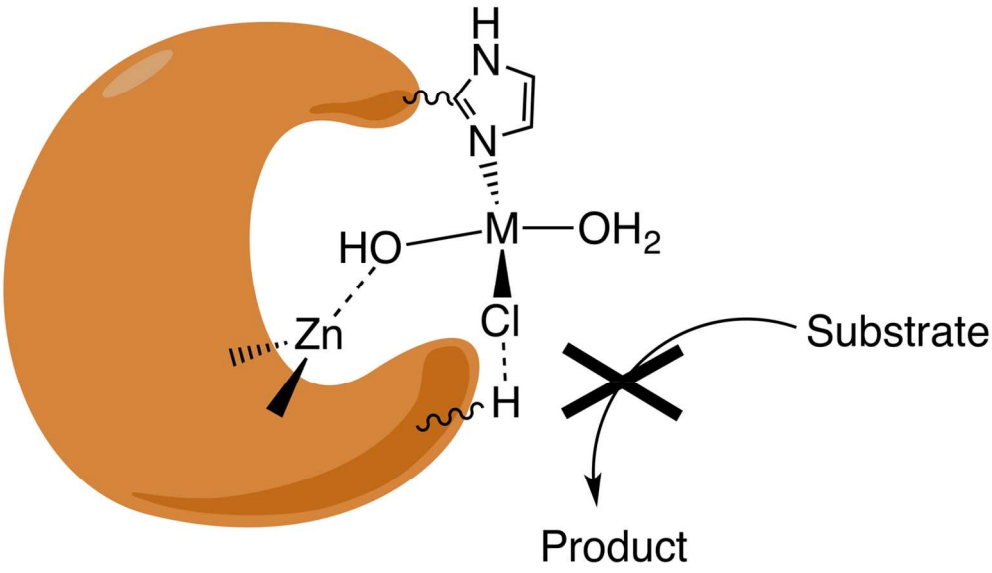
Metal complexes modulate the degradation of aggregation prone substrates by metalloproteases and can be used to tackle conformational diseases.



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Metal complexes and metalloproteases: targeting conformational diseases

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Abstract

In recent years many metalloproteases (MPs) have been shown to play important roles in the development of various pathological conditions. Although most of the literature is focused on matrix MPs (MMPs), many other MPs have been demonstrated to be involved in the degradation of peptides or proteins whose accumulation and dyshomeostasis are considered as being responsible for the development of conformational diseases, i.e., diseases where non-native protein conformations lead to protein aggregation. It seems clear that, at least in principle, it must be possible to control the levels of many aggregation-prone proteins not only by reducing their production, but also by enhancing their catabolism. Metal complexes that can perform this function were designed and tested according to at least two different strategies: i) intervening on the endogenous MPs by directly or indirectly modulating their activity; ii) acting as artificial MPs, replacing or synergistically functioning with endogenous MPs. These two different bioinorganic approaches are widely represented in the current literature and the aim of this review is to rationally organize and discuss both of them so to give a critical insight on these approaches and highlighting their limitations and future perspectives.

Keywords

Metal ions, neurodegenerative disease; cancer; Alzheimer's disease; metalloproteinase activity.

1. Metalloproteases and conformational diseases

Metalloenzymes comprise a vast family of proteins which are involved in many physiological processes fundamental for the life of cells and, more generally, of living organisms. There are many ways to describe and sort metalloenzymes discovered so far. One common and rational way is to group them according to the metal ion of the catalytic site that is in charge of the biological function of the metalloenzyme.[1] Following this method, MPs can be classified into zinc-, copper-, iron-, nickel-, or manganese-containing enzymes, where the specific inorganic features of the particular metal ion give to the enzyme its specific properties. In particular, zinc always stays in the +2 oxidation state as it cannot be oxidized nor reduced in biological conditions. For this reason, a large part of metalloenzymes functioning as metalloproteases (MPs) are zinc-containing MPs (ZnMPs). ZnMPs participate in biological reactions encompassing the degradation of all major metabolites (carbohydrates, lipids, nucleic acids, and proteins/peptides), and they can be classified according to their location[2] or main function.[3] In ZnMPs the metal ion has a catalytic role, and the mechanism for substrate degradation has been already thoroughly investigated. In the catalytic site, zinc is maintained in position by coordinating residues but it is also bound to water, thus generating a hydroxide ion that can attack the protein substrate (polarization assisted zinc water catalysis) or induce the formation of nucleophiles.[4]

Another method to sort MPs would be to identify which ones are involved with a particular disease. However, it is practically impossible to group univocally the various existing MPs and to associate them with a single disease, as most MPs seem to be involved in the development of several pathologies. In this perspective, matrix MPs (MMPs) certainly hold the sceptre of the multifunctional MP, as they play an important role in tissue remodeling, which is itself associated with various physiological and pathological processes such as angiogenesis,[5] morphogenesis,[6] tissue repair,[7] cirrhosis,[8] systemic sclerosis,[9] and metastasis.[10] Recent data suggested an active role of MMPs in the pathogenesis of aortic aneurysm, and a dysregulation of the balance between MMPs and their natural inhibitors (TIMPs) is also a characteristic of acute and chronic

cardiovascular diseases.[11] Indeed, the majority of MPs are involved in somewhat very different pathologies so that the only meaningful categorization can be carried out the other way around, that is, by listing all the MPs that are involved with a particular disease. In this review we will focus on conformational diseases (CDs), that, by definition, are diseases caused by mutations or other changes in the structure of specific proteins that lead to their aggregation and deposition.

Protein misfolding is an intrinsic aspect of normal folding within the complex cellular environment, and its effects are minimized in living systems by the action of a range of protective mechanisms that include molecular chaperones and quality control systems. MPs play a very important role in regulating and controlling protein misfolding. Indeed, in physiological conditions MPs contribute to lower the level of aggregation-prone proteins by degrading them if their concentration is above normal levels. However, several environmental factors can contribute to protein misfolding such as interaction with metal ions,[12,13,] interaction with small molecules,[14] pH changes,[15] etc.[16] Misfolded proteins have a tendency to aggregate to form a variety of species including the highly organized and kinetically stable amyloid fibrils, which is considered as the main culprit for the development of widespread pathological conditions such as Alzheimer's Diseases (AD), Parkinson Disease (PD), or prion diseases. Amyloid fibrils and their precursors (oligomers) appear to have adverse effects on cellular functions regardless of the sequence of the component peptide or protein. Table 1 lists the most widespread CDs that have been associated with the aggregation of a specific protein,[17,18] together with the MPs which are known to be involved either directly in the catabolism of the aggregation-prone protein, or indirectly with the disease. It is important to note that while some MPs seem to be specific for a particular disease, many others are involved in different diseases. Therefore, the fact that most MPs have multiple biological roles in living organisms makes the design of drugs targeting specific MPs a very challenging task, all the more to tackle one particular disease. For this reason, in addition to the efforts put by the scientific community into the design of specific MPs modulators, new therapeutic horizons have been opened by the use of metal

complexes (MCs), which have catalytic functions mimicking the ones of the endogenous MPs. Both approaches will be reviewed and discussed in the next sections.

2. Metal complexes as metalloproteases inhibitors

Because the activity of many MPs can be linked, directly or indirectly, to the development of specific diseases, the investigation of biomolecular mechanisms responsible for the alteration of enzymes activities,[19-22] and new analytical approaches that are able to assess enzymes kinetics,[23-26] have attracted much scientific interest in recent years. In particular, MCs are one of the most used molecules used to inhibit MPs, and, before reviewing the molecular details of these inhibitors, some definitions must be given. According to a recent review from Kilpin *et al.*[27] MCs inhibitors can inhibit a protein through *i)* only one of their ligands; *ii)* only the metal center; *iii)* both the metal center and one of the ligands. Most of the difficulties encountered by the scientific community consist in obtaining potent and selective MP inhibitors that are able to inhibit one given MP, for example by chelation of its zinc ion, but without interacting with metal ions of other MPs. Therefore, the MC design has to take into account both the issues of drug delivery[28] and coordination chemistry, i.e. the MC has to get the proper localization without undergoing collateral reactions and then has to be properly functionalized so to interact specifically with its selected target. The first metal complexes that were shown to inhibit MPs were "free" aluminium ions made by dissolving $AlCl_3$ in aqueous solution. Calpain, cathepsin D, trypsin and α-chymotrypsin[29] were shown to be inhibited by Al^{3+}, which is supposed to be able to substitute Mg^{2+} or Fe^{3+} ions in magnesium- or iron-dependent enzymes because of the similar ionic radius of these three ions. Such substitution is believed to be the cause of enzyme inhibition, and addition of the strong metal chelator EDTA sometimes was shown to revert inhibition.

Obviously, the absence of organic ligands that would make specific interactions with the binding pocket of the MP, rules out selectivity during enzyme inhibition by "free" metal ions. For this reason, various research groups have developed small organic molecules that show selective MP

inhibition by employing a zinc-binding group (ZBG) to bind the active site metal ion.[30] This was usually carried out by using a strong chelator such as hydroxamic acid. However, the use of hydroxamic acids ZBG has been shown to be limited by poor pharmacokinetics, low oral bioavailability, hydrolytic liability, and inadequate selectivity for zinc, which may account for most failures of those inhibitors in clinical trials.[31] In recent years, the search for new MPIs with major changes in the zinc(II) chelating groups appeared as a challenge for several research groups.[32,33] New ZBGs such as hydroxypyrimidinone[34] or 1-hydroxypiperazine-2,6-diones[35] have been explored. In this perspective, it is important to realize that the zinc(II) ion is a borderline Lewis acid that is able to interact with a variety of donor atoms such as sulfur, nitrogen, or oxygen. In order to design an effective and selective Zn-chelating drug it is therefore important to consider the thermodynamic ability of the metal chelator to coordinate the metal ions and to compete with the biological ligands that bind to the cofactor in the enzymatic environment.[36] The wide development of pharmacological inhibitors of MMPs (known as MMPIs) as potential anti-cancer agents began in the 1980s, but the clinical use of these agents in oncology has been brought to a crashing halt with the repeated failure of various MMPIs in multiple large-scale phase III clinical trials.[37] Recently, instead of the hydroxamic acid moiety, carboxylates have been proposed as a chelating agent that could function as a less strong and more selective MMPs inhibitor with a safer toxicity profile.[38] Other functional groups that may function as ZBGs are phosphinic acid, heterocycles, pyrimidinetrione, thiol diketopiperazine, carbamoyl phosphonate, and sulfone N-formylhydroxylamine.[94] The general active site of MMPs is a groove, usually presented horizontally across the protein with three un-primed sub-sites to the left of the coordinated zinc and three primed sub-sites to the right (S3-S2-S1-Zn-S1'-S2'-S3').[39] While most attempts to introduce selectivity have focused on the S1' channel, other groups also investigated the possibility of interaction with the S2' pocket.[40]

Very recently, ruthenium, osmium,[41] and platinum complexes able to inhibit MMPs through a non-competitive mechanism have also been reported,[42] for which the structure of the MCs bound to its

target MMP was even obtained.[43] The selectivity of such Pt complexes seems to be higher than that of classic organic inhibitors based on Zn chelation, since they seem to interact with specific amino acid residues of the binding pocket, which are different for each specific MMP. Figure 1 shows the structures of catalytic domains of MMPs and a model of their interaction with a specific platinum complex. The binding sites are in regions critical for the enzymatic activity, in particular, His224 for MMP-3 and Met404 for MMP-9 (both located in the S1' specificity loop), or Met87 for MMP-12, which ends up very close to the active site upon formation of a salt bridge between the N-terminal Phe84 and Asp237. It has been hypothesized by the authors that, after interaction of [$PtCl_2$(smp)] at primary anchoring sites on the MMPs surface, other binding events can take place, further stabilizing the inhibitor-enzyme interaction. For this reason, another MMP tested in this study, MMP-2, has a very low inhibition by [$PtCl_2$(smp)] because it lacks potential anchoring residues in such a favorable position to allow the concerted binding event.

Metal ions different from platinum have also been used as MMP inhibitors (see Figure 2). The ligands bound to the metal center sometime play an important role on the inhibition properties of the MC. For example, [Co(acacen)$(NH_3)_2$]Cl,[44] a complex with two substitutionally labile *trans* ammonia ligands, is almost ten times more potent as inhibitor of MMP-9 than [Co(acacen)$(Im)_2$]Cl, an analogue complex containing the substitutionally less labile imidazole ligands (Im). Binding of coordinating residues of the protein to the Co center must play a role in this case. By covalent attachment of the metal fragment with known MMP-inhibiting biphenylamide or biphenylsulfonate organic fragments, organic-inorganic bifunctional compounds showed 3- to 6-fold higher activities, with IC_{50} values down to 0.83 μM. Later on, the binding mechanism of [Co(acacen)$(NH_3)_2$]Cl to MMP-2 was investigated, and inhibition was shown to be irreversible, time-dependent, and to involve both the structural and catalytic histidine sites. According to kinetic analysis,[45] reversible formation of an enzyme-inhibitor complex occurs first (Figure 3a), followed by an irreversible reaction (Figure 3b). The kinetics of irreversible metal binding increased with temperature. Thus, metal binding to the protein involves structure loss of the protein upon binding ($\Delta S>0$) and ligand

loss before the histidine can bind ($\Delta H>0$). Overall, this study is one of the few quantitative analysis of the thermodynamic and kinetics of the irreversible inhibition of an MMP by a transition metal complex.

However, selectivity in the series of the tested MMPIs was not as good as anticipated, and specific MMPIs have proven to be extremely difficult to obtain. This is due to the fact that MMPs are quite similar structurally, particularly with respect to the catalytic site. In addition, there is evidence for crosstalk among the different protease classes that can possibly lead to compensatory mechanisms if one class is inhibited. Of course, even a highly specific inhibitor will be of no benefit if modulation of its target interferes with anti-angiogenic programs or immune cell-mediated tumor destruction. For this reason, the roles of MMPs in each cancer and disease setting must be very well understood. For example, inhibition of MMP-9 may be useful in preventing the priming of sites for metastatic formation, but once metastasis has occurred and has engaged an angiogenic program, MMP-9 inhibition could promote angiogenesis and lead to larger and more aggressive metastatic lesions.[46] Therefore, although there is ample evidence from animal models that MMPIs could effectively modulate disease progression, successful results regarding the use of MMPIs in human diseases are very scarce.[47] The reader interested with more detailed information on MMPI-based drugs that have been engaged clinical phase trials is advised to read the nice review on the subject by B. Fingleton (Ref. 47).

On the other hand, the development of inhibitors of other MPs different from MMPs have been more successful, and many drugs based on this approach are currently used in humans. For example, ACE inhibitors have been for a long time successfully applied in the treatment of hypertension and congestive heart failure[48] and, more recently, new MPIs which contemporarily inhibit the catalytic function of more than one MPs have been developed.[49] The benefit of this new class of dual or triple inhibitors (ACE/NEP; NEP/ECE and ACE/NEP/ECE) has been demonstrated but unfortunately only a few reached the clinical development stage.[50] In order to achieve the best results in term of specificity and inhibition activity, a thorough understanding of the relationship

between the structure and the properties of the designed MPIs is necessary. Moreover, the investigation of the inhibitor-MP complex is very useful in order to obtain crucial information on the structure-function relationship of the MPs themselves. For example, the detailed analysis of the crystal structure of the phosphoramidon-NEP complex shown in Figure 4 (PDB code 1DMT) allowed for explaining the enzyme's broad substrate specificity.[49] The main structural features observed in most selective NEP or ACE inhibitors are a ZBG (often a SH group), a pseudo-dipeptide unit, and a C-terminal carboxylate group. The ZBG has a strong impact on many factors such as inhibitor potency and selectivity. This is not only due to the affinity of the ZBG for the metal ion in the active site, but also to its hydrogen-bonding interactions with the protein and the van der Waals contacts. [51] Therefore, it is possible to optimize and design such interactions in order to find the best ZBG for a given metalloenzyme. In addition, specificity toward a MP can be obtained by adding a bulky portion in the P1' position of the MPI, so that, for example, NEP inhibition is stronger than ACE inhibition. Indeed, the S1' subsite of NEP is able to accommodate a larger group than the corresponding S1' subsite in ACE, determining a different affinity toward the MPI.[52]

In order to identify new chelating scaffolds for MPIs, another approach has also been applied that is based on the screening of low molecular weight compounds against drug targets.[32] Fragments are then linked or "grown" to generate potent leads, which can be further optimized to obtain improved solubility and pharmacokinetics via traditional medicinal chemistry. The main advantage of such approach is that active sites are more efficiently probed by small fragments that are not limited by steric constraints or hydrogen-bonding mismatches. For example, by following such method it has been demonstrated that substitution at either the 5- or 7-positions of the 8-hydroxyquinoline inhibitor produces potent leads against MMP-2, while derivatives at the 2- and 4-positions do not.[32] Finally, NEP inhibition has been also proposed as a possible treatment for type 2 diabetes,[110] but warning about the risk of reducing NEP activity is also raised by *in vivo* experiments that have clearly demonstrated that a reduction in NEP activity contributes to the development of AD.[53] For

this reason, activation instead of inhibition of NEP and other MPs has been also proposed as a possible therapeutic route to tackle a variety of CDs. Indeed, the reason why chelators have failed in the clinic is that therapeutic use of a metal chelator may result in indiscriminate binding of essential metal ions, leading to potentially harmful effects such as the scavenging of iron leading to anemia. Therefore, in the next section the use of MCs as possible positive modulators of MPs is also reviewed.

3. Metal complexes as metalloprotease activators

In Table 1 the various MPs involved with different CDs are listed. Although in the case of most CDs such as cancer, atherosclerosis, etc. a meaningful therapeutic approach is represented by the development of MPIs, in other widespread CDs such as AD, PD and Scrapie/Creutzfeldt-Jakob disease a positive modulation of some of the involved MPs could represent instead a valid therapeutic approach. Indeed, CDs are characterized by the accumulation and fibrillation of a misfolded protein, and increasing the activity of some of the MPs that are partly or totally responsible for the catabolism of this aggregation-prone protein may lower, in principle, the concentration of this protein.[54] However, as outlined above for NEP, modulating the activity of MPs involved in different biomolecular functions can also be detrimental to health. Another example is indeed represented by ACE, the inhibition of which is commonly targeted in elderly populations because it plays a central role in the regulation of blood pressure and hypertension. However, as ACE is able to degrade Aβ, an up-regulation of this enzyme has been proposed as a possible therapeutic route for AD, while its inhibition enhances Aβ deposition in the brain and is therefore potentially dangerous.[55]

In this scenario, MCs were not only proposed as MPs inhibitors (see previous section), but also as MPs activators. In the latter case, the up-regulation of MPs usually occurs through a multistep biomolecular process, rather than by direct interaction of the MC with the enzyme. The most successful application of a chelating ligand as an activator of MPs for therapeutic treatment of a CD

is probably the use of PBT2. Indeed, this drug has completed phase IIa clinical trials and is currently in phase IIb for the treatment of AD[56,57] and phase IIa against Huntington Disease. Although PBT2 and its predecessor clioquinol (see Figure 5) have been initially designed as metal chelators to target the ascertained metal ion dyshomeostasis involved in AD,[58] it has been later recognized that their success as modulators of the Aβ protein level is due to their ability to work as ionophores. Indeed, they are able to shuttle metal ions inside the cell, i.e., to re-establish their correct spatial distribution, which seems to be one of the major problems in AD rather than a simple alteration of the metal ion levels.[59] Once the metal ions are brought back inside the cell, it has been demonstrated that they activate PI3K (phosphoinositide 3-kinase), which leads to the downstream phosphorylation of GSK3 (glycogen synthase kinase 3) and potentiation of the MAPK (mitogenactivated protein kinase) JNK (c-Jun N-terminal kinase). The stimulation of this pathway culminates in up-regulation of cellular MP activity and degradation of extracellular Aβ.[60,61] This delicate mechanism of action is very much dependent on the coordinating ligand (PBT2) that functions as a metal "chaperone". Therefore, extensive screening studies have been carried out in order to decide which ligand has the best impact on metal uptake, MAPK signalling pathways, and Aβ levels.[62] Indeed, an important feature of these compounds is their not-too-high affinity towards metal ions. Such moderate metal affinity allows them to remove metals from extracellular amyloid aggregates without chelating metals from the catalytic sites of MPs.[63] Therefore, in our opinion this laborious but successful study represents an important milestone in the field of applied medicinal bioinorganic chemistry,[64] which demonstrates the potentiality of MCs in tackling so far incurable CDs.

An indirect up-regulation of MMPs has even been reported to occur with the well-known anti-tumor drug cisplatin.[65] In the investigated case of thyroid PC Cl3 cells, cisplatin seems to provoke an oxidant-induced MMP-2-dependent EGFR transactivation responsible for the induction of cell apoptosis, a process ascribable to the intracellular signaling of PKC-e and MAPK/p38. Overall,

these biological studies highlight the intricate character of MC/MP interaction: even in cases when a direct chemical interaction does not occur, indirect biological interaction is possible.

Finally, it is important to highlight that even endogenous MCs have been reported to stimulate MPs activity.[66] Glycyl-L-histidyl-L-lysine (GHK) is a tripeptide which was isolated from human plasma for the first time in 1973.[67] Either alone or in combination with copper(II) ions it was initially used to make hair- and skin-care products. More recently it has also been proposed as a possible therapeutic agent against age-associated neurodegeneration and cognitive decline.[68] Interestingly, its copper(II) complex seems to be involved in wound remodeling, and the mechanism of action has been demonstrated to be the modulation of MMP-2 expression.[66] Another MC based on zinc(II) ions bound to a naturally occurring ligand, kinetin (N6-furfuryladenine), has also been demonstrated to change noticeably the pro-MMP-2/MMP-2 ratio towards a higher amount of mature MMP-2, therefore modulating the inflammatory response in THP-1 cells.[69] Overall, although the above-mentioned examples of MCs used as MPs activators show the possibility to develop new therapeutic agents, the prediction of their efficiency *in vivo* is very difficult because of the indirect mechanisms of modulation of MMPs by MCs, which is often unveiled *a posteriori*. For this reason, a more versatile approach that, on paper, seems to have a more predictable output, is presented hereafter, which consists in the use of MCs as artificial MPs.

4. Metal complexes as artificial metalloproteases

The lack of specificity of MCs as MPIs spurred another area of investigation in the field of bioinorganic chemistry, that is, the development of artificial proteases capable of degrading specific proteins involved in CDs. Providing that reasonable target specificity could be attained, such molecules could represent a new and promising class of molecular therapeutics.[70] The major advantage of this approach is the catalytic nature of these drugs, which would allow for using lower drug dosages and, consequently, for minimizing side effects. In the literature, very recent and comprehensive reviews on this subject have been published,[70-72] so that we will only indicate the

main features allowing a MC for appearing as an artificial MP, and elucidate the biomolecular mechanisms of action of the most commonly used artificial MPs.

First of all, it is important to highlight that the selective hydrolysis of peptide bonds by artificial MPs is a very challenging task. Indeed, despite their name "peptides" comes from the Greek πεπτοζ – "digestable", this kind of bonds are rather unreactive if compared for examples with esters, as they have a very long half-life in solution (at pH 4–8 their spontaneous hydrolysis would take several hundred years at room temperature!). For this reason, nature has equipped living organisms with MPs, which are highly specialized enzymes that specifically interact with the protein that has to be degraded. It has been recently recognized that the microenvironment of the active site of the various MPs is the crucial feature that determines the height of the energetic barrier for the hydrolytic cleavage of a peptide bond.[73] This energy barrier eventually determines the hydrolysis rate, and so far artificial MPs are well below natural MPs performances. However, artificial MPs have some advantages on natural ones, which spur the scientific community to develop efficient artificial MPs as therapeutic drugs in many of the above-listed CDs. Such advantages are: i) a better stability to heat; ii) a better compatibility with organic solvents; iii) their applicability to abiotic reactions; and iv) sometimes a better selectivity.[74] As this area progresses, research interests now focus on artificial MPs that can solve biological problems that cannot be solved by natural enzymes. Indeed, the medicinal application of natural enzymes is limited because of their special requirements, notably their sensitivity to temperature and pH. Also, many natural MPs are poorly selective and cleave peptide bond indiscriminately, to give many unwanted short fragments. On the contrary, artificial MPs can in principle be designed to be highly specific toward any particular target. Moreover, artificial MPs may also provide a new method for the detoxification of many soluble protein toxins.

An artificial MP should have key features to be an efficient drug against a CD. A first requirement is to have at least two coordination sites occupied by weak ligands that can be readily replaced, one for anchoring to the side chain of the amino acid in the peptide, and a second one for interaction

with the nearby peptide bond to hydrolyse.[75] The remaining coordination sites should be occupied by one or several polydentate ligand(s) that clearly define a stable coordination sphere for the metal ion. The perfect candidate for the metal ion should be a strong Lewis acid with limited redox activity in biological conditions in order to avoid side reactions such as the production of reactive oxygen species (ROS). In any case, the metal ion should be as oxophilic as possible in order to interact with an amide carbonyl oxygen in the peptide backbone, which activates the amide quaternary carbon toward nucleophilic attack (Figure 6a). Simultaneously, oxophilic metal ions may deliver a hydroxide nucleophile to cleave the amide bond (Figure 6b). In addition to the metal complex, a targeting domain should also be present in the artificial MP to control the localization of each reactive catalyst to the protein target.[76] In some cases, an aldehyde group has been employed as the binding site of artificial proteases because of its ability to form imine bonds with the ε-amino groups of Lys residues exposed on the surface of proteins. Since the imine bonds are readily hydrolysed, the artificial protease equipped with an active site containing the aldehyde group may be able to form complexes with a variety of proteins reversibly.[77] More recently, a series of amyloid peptide-binding groups were covalently attached to Co(III) or Cu(II) complexes to obtain catalytic cleavage of the peptide responsible for AD (Figure 6c). In one case, apoptosis of INS-1 cell in the presence of preformed polymeric aggregates of h-IAPP was even inhibited.[72,78] Other strategies can be mentioned here. In order to increase the intrinsic proteolytic activity of a metal centre it is also possible to alter the polarity of its microenvironment.[79] For example, it was found that the proteolytic activity of the Cu(II) complex of 1,4,7,10-tetraazacyclododecane (cyclen) is enhanced significantly when attached to crosslinked polystyrene.[77] Another mechanism of action reported in the literature consists in the abstraction of an α-carbon hydrogen atoms of the peptide backbone by hydroxyl radicals and other ROS generated by a redox-active metal center. Subsequent degradation of the NH-Cα and Cα-C(O) bonds yields multiple, fragmented peptide products.[80] However, as it was mentioned above, in the latter case damage of the protein could be a problem. An interesting but unique example also reports the attachment of an iron(III) peptide-cleaving complex to

phospholipids, which allowed for cleaving specifically integral membrane proteins (Figure 6d).[81] Finally, sequence specific peptide hydrolysis can be achieved if the metal ion is capable of coordinating to anchoring side chains in amino acid residues such as cysteine, aspartate, histidine, or methionine. One for all, it is instructive to mention the results reported by A. Erxleben[82] showing the reaction of Cp_2MoCl_2 with cysteine-containing di- and tri-peptides, where the coordination of the Cp_2Mo^{2+} unit to the thiol group of X-Cys-Y peptides assists the release of the amino acid at the carboxyl end of the cysteine residue.

4. Conclusions and perspective

In the current bioinorganic literature concerning CDs, most attention is given to the interaction between metal complexes and the proteins whose misfolding or dyshomeostasis is considered as responsible for the development of the disease. On the other hand, although it has been recognized that MPs play an important role in the development of many CDs (see Table 1), the inhibiting properties that MCs might have towards MPs and the therapeutic consequences of those inhibitions properties, have not received, in our opinion, enough attention. Meanwhile, although MCs have been considered for a long time as possible drugs for CD, their mechanism of action and the question of whether they interact with MPs, have only been recognized at a second, much more recent glance (see the case of clioquinol or cisplatin discussed above). The possibility to modulate MPs activity by MCs opens fascinating therapeutic strategies, in particular because several MPs play a role in different CDs. Thus, compounds that are active in one CD might be active as well in other CDs. Of course, this interpenetration of MPs inhibition also represents a challenge for medicinal chemistry, as ultimately only drugs that show a selective therapeutic action in a single type of disease can be retained. The few published cases in which MCs have been shown to act either as direct inhibitors of endogenous MPs, or as indirect activators of MPs, can be taken as a proof-of-concept that inorganic complexes can be complementary to organic inhibitors in

modulating MPs activity. Meanwhile, the more numerous cases of artificial MPs that could substitute malfunctioning MPs and lower the concentration of misfolded, aggregate-prone proteins or peptides, have already opened new routes for bioinorganic chemistry. Overall, only a better understanding of the action of MPs in CDs, and of course *in vivo* data on the selectivity of existing and future metal-containing MP inhibitors, will allow for developing new and efficient drugs for so far incurable diseases.

6. Acknowledgements

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Tables

Conformational Disease	Protein	MPs involved	Ref.
Hypercholesterolaemia, Atherosclerosis	low-density lipoprotein receptor	MMP-12	83
Cystic fibrosis	cystic fibrosis trans-membrane regulator	MMP-9, MMP-13	84-86
Phenylketonuria	phenylalanine hydroxylase	Not identified	87
Huntington's disease	huntingtin	MMP-9	88
Marfan syndrome	fibrillin	MMP-2, ADAMTSL6β	89,90
Osteogenesis imperfecta	procollagen	BMP1	91
Sickle cell anaemia	hemoglobin	MMP-2, MMP-9	92
Scurvy	collagen	MMP-1	93
Alzheimer's disease	β-amyloid, presenilin	NEP, IDE, ECE-1, ECE-2, ACE, BACE2, BACE1 MMPs, PreP. plasmin, APH, MBP, CatB, CatD, the proteasome	94
Parkinson's disease	α-synuclein, neuromelanin, lactoferrin, ferritin, ceruloplasmin, bivalent cation transporters	MMPs, ADAMs,	94-96
Scrapie/Creutzfeldt-Jakob disease	prion protein, ferritin	ADAM 8, ADAM9, ADAM 10	94,97-99

Familial amyloidoses	**transthyretin/lysozyme**	**MMP-9**	**100**
Retinitis pigmentosa	**rhodopsin**	**ADAM-9**	**101**
Cataracts	**Crystallins**	**MMPs, ADAM, ADAMTS**	**102,103**
Cancer	**p53**	**MMPs, IDE, ADAMs**	**104,105**
Friedreich's ataxia	**Frataxin, aconitase**	**MPP**	**106**
Progressive supranuclear palsy	**α-Synuclein**	**MMP-1, MMP-9**	**107**
Wilson's disease	**Ceruloplasmin, Wilson's protein**	**MMP-2, MMP-12**	**108**
Type II diabetes	**Insulin, amylin**	**IDE, NEP**	**109,110**
Carotid atherosclerosis	**Proteins in vessel walls**	**MMP-9, MMP-12**	**111,112**
Lewy-body dementia	**α-Synuclein**	**MMPs**	**113**
Familial amyotrophic lateral sclerosis	**Superoxide dismutase 1**	**MMP-3, MMP-9**	**114,115**

Table 1: aggregation prone proteins and MPs involved with various CDs

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Figures and Tables Legends

Table 1: Representative conformational diseases. The aggregation-prone protein and the MPs capable of degrading the latter are also reported. Readapted from ref. 17.

Figure 1: The ligand smp (bottom) and the structures of catalytic domains of MMPs and model of their interaction with [$PtCl_2$(smp)]. Ribbon representation of the structures of catalytic domains of MMP-2 (PDB ID 1HOV), MMP-9 (1GKC), MMP-3 (1SLN), and MMP-12 (theoretical model from reference 42 with [$PtCl_2$(smp)]). Helices and strands are coloured in grey. The zinc atoms are shown as cyan spheres and the calcium atoms as magenta spheres. The catalytic and structural zinc sites as well as the loops S and S1' are indicated on the structure of MMP-2. Fully conserved His and Met residues are shown as yellow sticks, while non-conserved His and Met residues are shown as blue and green sticks, respectively. The smp ligand of [$PtCl_2$(smp)] bound to MMP-12 is shown in red and the platinum atom as an orange sphere.

Figure 2: MMP inhibitors based on a) cobalt(III) and b) platinum(II) MCs. [Co(acacen)$(NH_3)_2$]Cl: R=CH_3, Y=H, X=NH_3.

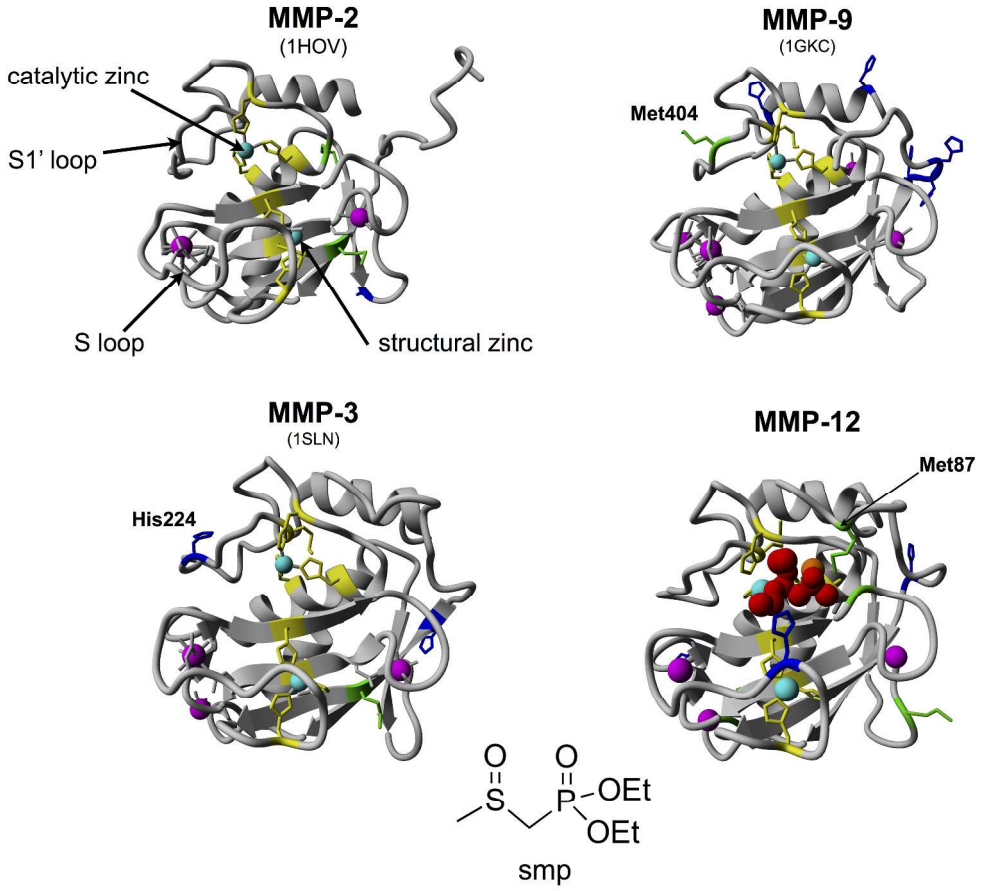
Figure 3: Metal-based MMP inhibitors: reversible inhibition with an organic, zinc-binding moiety can occur first (a), followed by irreversible coordination of an MMP aminoacid residue to the inhibitor metal center (b). In the final, irreversible complex additional secondary interactions such as H-bonding, π-π interaction, etc. can occur between the secondary ligands coordinated to the inhibitor metal ion, and either nearby residue of the MMP, or the catalytic zinc ion itself.

Figure 4: Zoom on the crystal structure of phosphoramidon-NEP (PDB code 1DMT). The inhibitor phosphoramidon in the centre of the molecule is in stick-and-ball form (color code: orange=C,

magenta=N, red=O, and yellow=P) and the zinc ion is represented by a cyan sphere. The coordinated His and Glu residues are colored in blue and green, respectively.

Figure 5: Two metal chelators in clinical trials for the treatment of neurodegenerative diseases. Reprinted with permission from Ref. 64.

Figure 6: Artificial metalloproteases. Two mechanism for peptide cleavage: a) chelation of peptides followed by nucleophilic attack of OH^-; or b) coordination of peptides followed by nucleophilic attack of a metal-bound OH^- ligand. c) Amyloid beta peptide-targeted artificial metalloprotease (readapted from Ref. 78, peptide taken from PDB 2LFM). d) Membrane-bound artificial metalloprotease for cleavage of integral membrane proteins.



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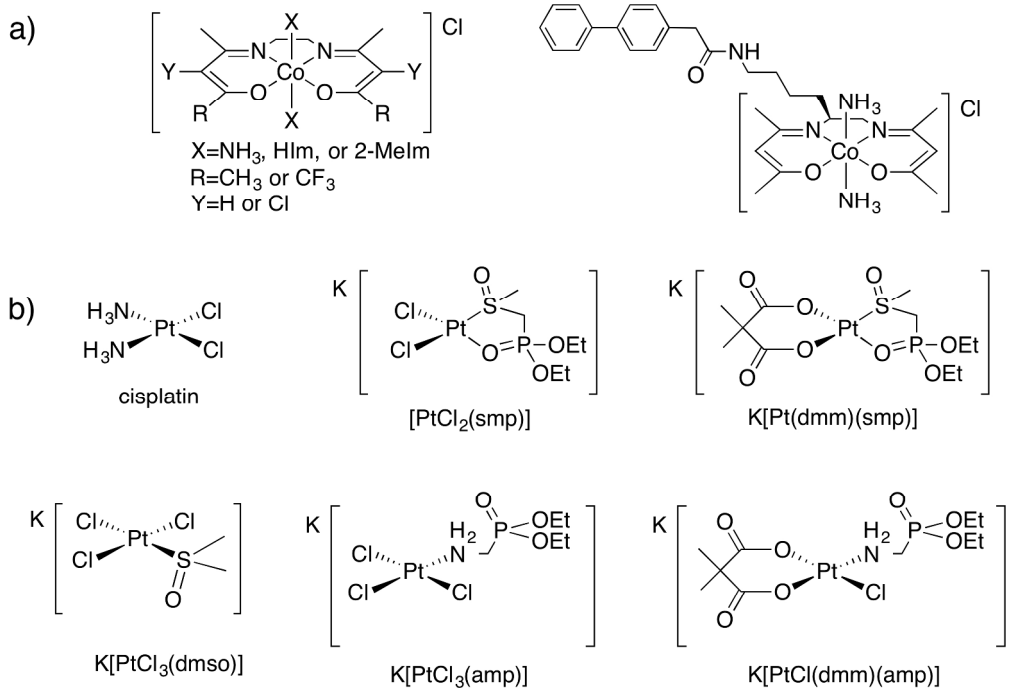

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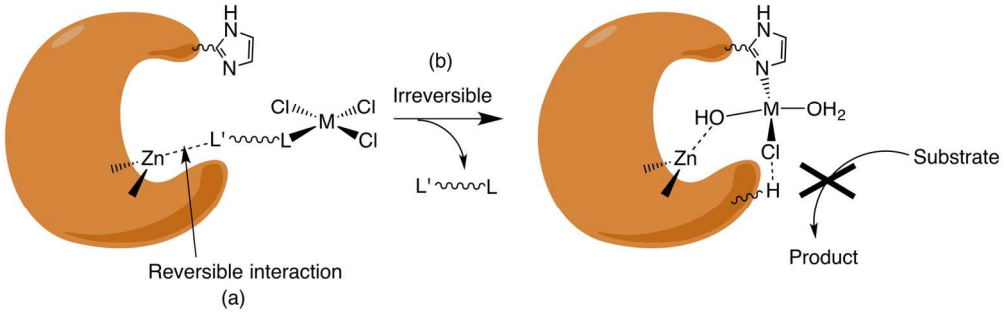

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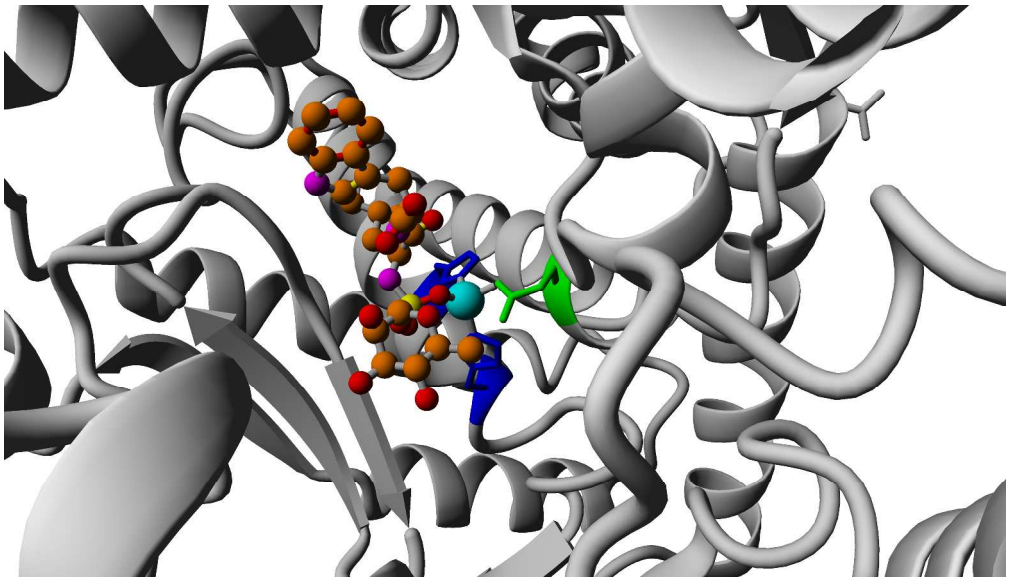

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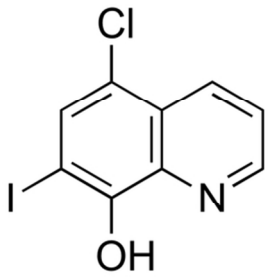
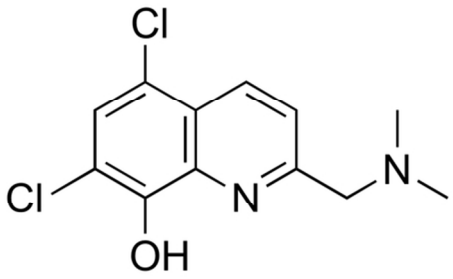


clioquinol



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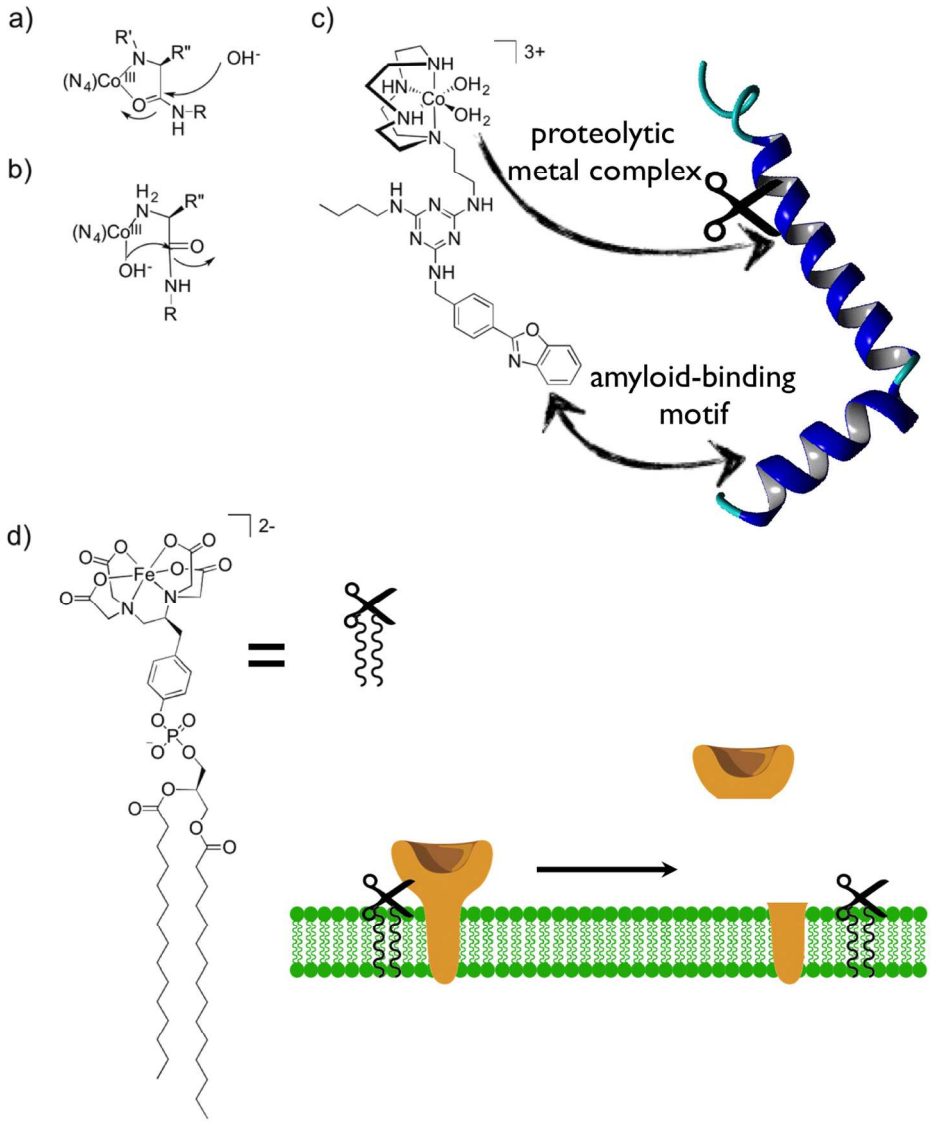

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