ACCOUNTS of chemical research

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Mysteries of Metals in Metalloenzymes

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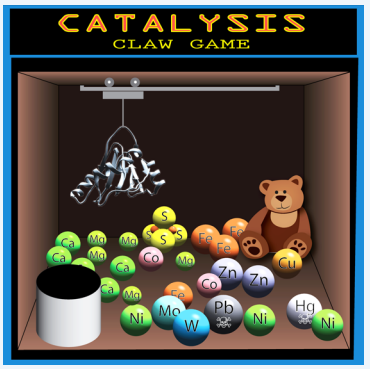
CONSPECTUS: Natural metalloenzymes are often the most proficient catalysts in terms of their activity, selectivity, and ability to operate at mild conditions. However, metalloenzymes are occasionally surprising in their selection of catalytic metals, and in their responses to metal substitution. Indeed, from the isolated standpoint of producing the best catalyst, a chemist designing from first-principles would likely choose a different metal. For example, some enzymes employ a redox active metal where a simple Lewis acid is needed. Such are several hydrolases. In other cases, substitution of a non-native metal leads to radical improvements in reactivity. For example, histone deacetylase 8 naturally operates with Zn^{2+} in the active site but becomes much more active with Fe^{2+}. For β-lactamases, the replacement of the native Zn^{2+} with Ni^{2+} was suggested to lead to higher activity as predicted computationally. There are also intriguing cases, such as Fe^{2+}- and Mn^{2+}-dependent ribonucleotide reductases and W^{4+}- and Mo^{4+}-dependent DMSO reductases, where organisms manage to circumvent the scarcity of one metal (e.g., Fe^{2+}) by creating protein structures that utilize another metal (e.g., Mn^{2+}) for the catalysis of the same reaction. Naturally, even though both metal forms are active, one of the metals is preferred in every-day life, and the other metal variant remains dormant until an emergency strikes in the cell. These examples lead to certain questions. When are catalytic metals selected purely for electronic or structural reasons, implying that enzymatic catalysis is optimized to its maximum? When are metal selections a manifestation of competing evolutionary pressures, where choices are dictated not just by catalytic efficiency but also by other factors in the cell? In other words, how can enzymes be improved as catalysts merely through the use of common biological building blocks available to cells? Addressing these questions is highly relevant to the enzyme design community, where the goal is to prepare maximally efficient quasi-natural enzymes for the catalysis of reactions that interest humankind.



Due to competing evolutionary pressures, many natural enzymes may not have evolved to be ideal catalysts and can be improved for the isolated purpose of catalysis *in vitro* when the competing factors are removed.

The goal of this Account is not to cover all the possible stories but rather to highlight how variable enzymatic catalysis can be. We want to bring up possible factors affecting the evolution of enzyme structure, and the large- and intermediate-scale structural and electronic effects that metals can induce in the protein, and most importantly, the opportunities for optimization of these enzymes for catalysis *in vitro*.

From the isolated standpoint of catalysis, the general principles that should govern the construction of metal-containing active sites in enzymes are fairly clear. For example, a metal playing the role of a Lewis acid needs to have an appropriate charge and coordination geometry, and the quality of its interactions with a substrate can be described via the HSAB theory. Redox centers must have appropriate reduction potentials and orbital populations that result in minimal structural rearrangements upon charge transfer (in order for charge transfer to be efficient, in accord with Marcus theory). However, understanding the evolutionary optimization of enzymes goes beyond following these chemistry principles. Metalloenzymes exist in a cellular environment where they have to remain stable and properly structured and have the ability to find and bind the needed metals. Metals are predominantly toxic, scarce, or too reactive to be readily available without an elaborate metal-storage and delivery mechanism. Cells are dependent on metal trafficking pathways that bypass metal detoxification steps and allow metals to be utilized by some enzyme in the cell.[2,3] Metals can also compete for binding sites with other cations of comparable size, making concentration and selection regulation necessary for cell life. The cell must overcome challenges imposed by the relative stability of complexes formed by divalent transition metals, also known as the Irving–Williams series.[4,5] One way to overcome this is through localized folding within the cell, passing the enzyme through compartmentalized locations where specific metal ions exist.[6] Additionally, catalytic processes in the cell are tuned to proceed at a physiologically convenient rate, that is, not too fast and not too slow. Evolution takes care of all aforementioned aspects at once, thus imposing multiple constraints on enzymes as catalysts. Phenomenologically, this means that understanding enzymes requires a holistic approach at the cellular or organismal level, and they should be improvable for catalysis *in vitro*. Resultantly, so far, nature is the only true holder of the

Received: June 19, 2014
Published: September 10, 2014

   dx.doi.org/10.1021/ar500227u | *Acc. Chem. Res.* 2014, 47, 3110–3117

key to enzymology. Examining natural metalloenzymes through the looking glass of a chemist, we sometimes find surprises. In this Account, we aim to highlight a few cases of perplexing choices for metals in metalloenzymes and unexpected effects of metal replacement on the activity of enzymes to illustrate this conundrum. For some systems, the explanations can be found in the structures and catalytic mechanisms, but some cannot be explained without the involvement of the bigger, alas poorly understood picture. Our goal herein is to provide not a full review of the field but hopefully a diverse set of provocations.

STRANGE OR SUBOPTIMAL CHOICES FOR CATALYTIC METALS

Metal-dependent hydrolases, such as peptidases, phosphatases, amidases, and ATPases, catalyze hydrolysis. They contain one or two metal centers that activate the nucleophile (typically H_2O or OH^-), the substrate, or both for the key step in the reaction, nucleophilic attack. The metals play the simple role of a Lewis acid. Many hydrolases use Zn^{2+} as the metal of choice, but some are inhibited by Zn^{2+} and activated by Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, or Ca^{2+}. Furthermore, sometimes enzymes hydrolyze very similar substrates but by different metals. From the standpoint of bioavailability, the recruitment of redox-active metals (e.g., iron, whose relative abundance in the Earth's crust is much higher than in living organisms) as Lewis acids seems wasteful. On the other hand, it is possible that Zn^{2+} is so frequently used only because of its easy availability.[7] The natural question arises, have enzymes evolved to utilize not the most catalytically optimal metal but the most readily available one? The degree to which proteins are selective to these divalent metals is also intriguing.

One interesting monometallic hydrolase where the trade-off between activity and availability can be observed is histone deacetylase 8 (HDAC8). It participates in the regulation of gene expression. HDACs and their complements, histone acetyltransferases (HATs), modify the lysine residues of histones by removing or adding an acetyl moiety to the ε amino group (Figure 1A).[8,9] Histones are the primary protein component of chromatin, the DNA and protein "package" that fits inside a cell nucleus. Acetylated histone lysines are associated with an open chromatin structure that permits DNA transcription, while deacetylated lysines are associated with tightly packed DNA that is resistant to transcription.[10] Thus, deacetylation is correlated with gene silencing.

HDAC8, the best-characterized HDAC, is generally classified as a zinc-dependent enzyme even though its Asp_2His metal binding site is considered unusual for zinc (Figure 1B).[11] Indeed, experimental studies[11] demonstrate that HDAC8 has a 10^6-fold greater affinity for Zn^{2+} over Fe^{2+}. However, the Fe^{2+}-containing enzyme is significantly more catalytically active, with a k_{cat}/K_M value almost three times that of the Zn^{2+} variant (2300 ± 160 M^{-1} s^{-1} for Fe^{2+} vs 800 ± 50 M^{-1} s^{-1} for Zn^{2+}).[12] Depending on experimental conditions, HDAC8 either does not bind Fe^{3+} or suffers a >85% loss in activity when Fe^{2+} is oxidized to Fe^{3+}.[11,12] The question of which metal the enzyme contains *in vivo* remains contentious, since typical methods used to extract and purify the enzyme could oxidize Fe^{2+}, resulting in its replacement with Zn^{2+}.[11] Additionally, despite the greater affinity for Zn^{2+}, typical intracellular concentrations of Fe^{2+} are considerably higher (0.2–6 μM for Fe^{2+} vs 5–400 pM for Zn^{2+}), which could allow iron to out-compete zinc in the absence of nonthermodynamic considerations (e.g., metallochaperones).[11]

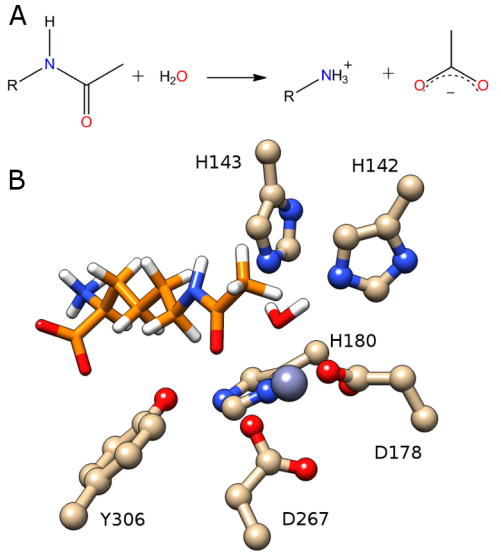


Figure 1. (A) Reaction catalyzed by HDACs, including HDAC8. (B) Structure of HDAC8 active site, based on crystal structure with PDB accession code 2V5W. The acetylated lysine substrate is shown in orange. The protein coordinates a metal ion via an Asp_2His binding pocket.

Of further interest is the even higher catalytic activity of Co^{2+} ($k_{cat}/K_M = 7500 \pm 300$ M^{-1} s^{-1}, a 9-fold increase over the zinc-containing enzyme).[12] With the exception of vitamin B_{12} (cobalamin) cofactors, cobalt-containing enzymes are exceedingly rare, and the intracellular concentration of cobalt is low.[13] The fact that HDAC8 has evolved to use iron or zinc may represent a compromise with metal bioavailability. Another intriguing possibility is that evolutionary pressures may select for a more limited catalytic activity. An overly active HDAC could be too proficient at silencing genes and hiding them from transcription factors. HDACs coevolved with their complement HATs, and cells must maintain a balance between their respective activities for optimal function.

Bimetallic hydrolases also exhibit unusual metal-dependent functionalities. Here, we focus on bimetallic amidases, ureases and β-lactamases. Urease is present in plants, bacteria, and yeast and catalyzes the hydrolysis of urea.[14] Its function is widely encompassed in medical and agricultural settings, not only in nitrogen mineralization but also as the main virulence factor in many pathogens, such as *Helicobacter pylori*.[15] Urease was the first enzyme characterized to contain Ni in its active site, a puzzling discovery[16] since the job of a Lewis acid can be done easily by a non-redox metal.[7,17]

In comparison, metallo-β-lactamase is an amidase that employs two Zn^{2+} ions. Metallo-β-lactamases hydrolyze β-lactam antibiotics, such as penicillin, and are the primary cause of antibiotic resistance in bacteria.[18,19] Thus, urease and metallo-β-lactamase both hydrolyze amides, according to the general scheme shown in Figure 2A, and both contain a dimetal active site with a very similar arrangement of ligands (Figure 2B,C). What is the reason behind the selection of Zn^{2+} or Ni^{2+}?

Considering the natural substrates catalyzed by the respective enzymes, urea has a resonance stabilized structure that makes it intrinsically more difficult to hydrolyze compared with the strained four-membered ring found in β-lactam antibiotics. In a purely computational study,[20] we employed urease to hydrolyze β-lactam and found that the reaction proceeds easily. Conversely, β-lactamase is unable to hydrolyze resonance-

dx.doi.org/10.1021/ar500227u | Acc. Chem. Res. 2014, 47, 3110–3117

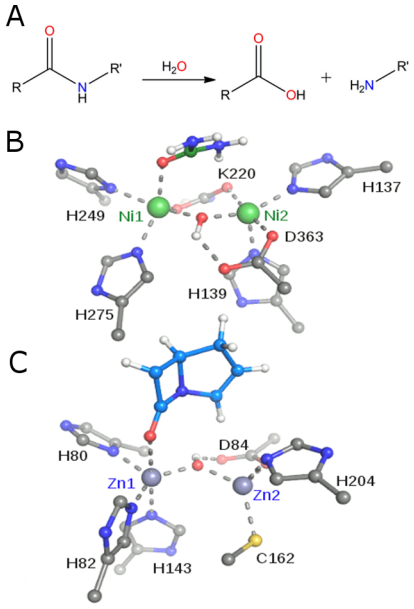


Figure 2. (A) Amide hydrolysis catalyzed by metal-containing amidases. The active sites of (B) urease and (C) metallo-β-lactamase with their respective substrates share similar ligand coordination environments yet contain different metals.

stabilized urea. From a structural standpoint, the active site of urease is probably suboptimal for binding β-lactam drugs. Therefore, we substituted the two Zn^{2+} ions in β-lactamase with Ni^{2+} ions. The Ni-substituted β-lactamase is predicted to catalyze the hydrolysis of the β-lactam ring with an efficiency surpassing that of natural di-Zn β-lactamase.[20] This suggests that the use of Zn in β-lactamase does not provide maximal possible efficiency for the enzyme. Zinc gives a performance that is satisfactory for biological purposes but could be improved via substitution with Ni. The use of Zn instead of Ni in β-lactamase could be dictated by other factors in the cell, such as the toxicity or lower availability of Ni. Computationally derived mechanisms have the ability to elucidate these metal-dependent features of metalloenzyme catalysis.

Another interesting example is a urease found in *Heliobacter mustelae*, a gastric pathogen in ferrets that employs two Fe^{2+} ions in the active site (Figure 3).[21,22] Although the Fe-containing urease is less active then the native Ni-containing urease, its activity is adequate for the survival of the pathogen in the ferret's low-nickel gastric environment. This could represent an instance of evolutionary adaption of the enzyme for its specific niche, surviving a low pH and low Ni environment. Carter et al.[23] show that this Fe-containing urease could be substituted with Ni under specific low Fe level and high Ni level *in vitro* conditions. The Fe-containing urease differs from the nickel urease by 92 residues, which are hypothesized to map to a region near the active site that influences metal loading.

Through these examples, we infer that hydrolases exemplify the interplay between most proficient catalysis and other evolutionary pressures. Metal use in enzymes is not straightforward and therefore not always optimal considering catalytic properties alone. Taking this message one step further, we can suggest that some natural enzymes could be improved for catalysis *in vitro* through metal replacement.

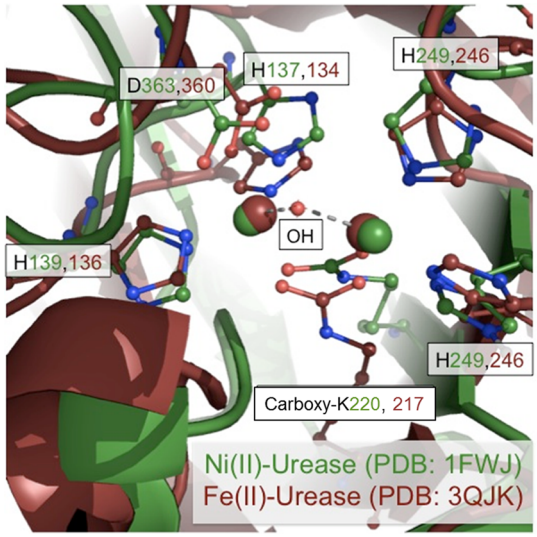


Figure 3. Overlay of Ni^{2+} and Fe^{2+} urease reveals essentially identical active site ligand environments.

ENZYMES THAT USE SEEMINGLY UNSUITABLE REDOX METALS

In view of the sensitivity of enzymes as simple as hydrolases to the identity of the catalytic metal ion, it would seem unreasonable to expect redox enzymes to swap metals: a specific reduction potential is required for each reaction. Nevertheless, there are cases when metal-replacement works, specifically in situations where the needed metal is, or was, scarce. Through a variety of strategies, enzymes tune the reduction potential of one metal to behave more like another, with a varying degree of success. Few such mechanisms are currently understood, and likely many of them are still undiscovered.

One example is **Mn in place of Fe in ribonucleotide reductases (RNRs)**. Most organisms, including humans, depend on Fe for a variety of enzyme-facilitated processes, from electron transport to cell replication. However, in the presence of large amounts of H_2O_2, that is, in conditions of extreme oxidative stress, Fe^{2+} can be oxidized and removed from enzymes through Fenton chemistry.[24] Most organisms have developed peroxide scavenging enzymes, catalases and peroxidases, that quickly remove H_2O_2 from the cells.[25] However, it was recently discovered that some organisms have a different means of survival in conditions of oxidative stress: they employ Mn instead of Fe in certain crucial enzymes.[26,27] One prominent enzyme in which this Mn−Fe trick was observed is bacterial RNR, specifically binuclear Fe_2^{2+} RNRs. These redox enzymes catalyze the conversion of ribonucleotides into deoxyribonucleotides (Figure 4A)[28,29] and thus are needed for the replication of DNA.

There is an interesting Mn-vs-Fe chemistry that takes place in the β-subunit of this protein, shown in Figure 4B,C. The β-subunit is responsible for the production of the tyrosyl radical, the oxidant of the cysteine residue located 35 Å away from the α-subunit, where the reaction in Figure 4A takes place. Some bacteria, such as *Streptococcus sanguinus*, and *Escherichia coli*, have the NrdAB RNR, with a partially understood mechanism of action. Additionally, these organisms have a homologue of NrdAB, called NrdEF, whose function has long remained unclear. A series of recent works[27,31] revealed that NrdEF becomes active in conditions of oxidative stress. NrdEF

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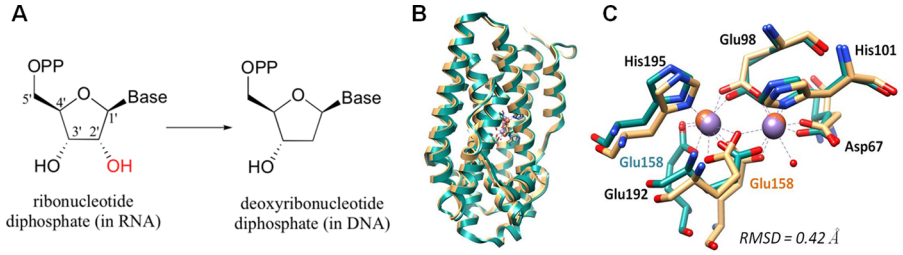

Figure 4. (A) RNR reaction. Di-Fe RNR with the native Fe and substituted with Mn: (B) secondary structure overlays very closely, but (C) there are shifts in the immediate coordination of the metals, especially the residue Glu158.[30]

preferentially binds Fe in the presence of excess Fe, but in this form, its activity is insufficient to drive DNA replication.[27] In low Fe conditions, it binds Mn and gains a 5-fold increase in activity. In contrast, NrdAB is an incompetent catalyst in the Mn-form. The site in Figure 4C can function as di-Mn, or Fe−Mn.[32,33]

What are the factors that make one RNR active and the other inactive with Mn as a cofactor? Electronic differences between the two metals and the subtle differences in the geometries of the Fe and Mn active sites (Figure 4B,C) may lead to differences in the reduction potential.[27,31] Overall, structures of NrdEF with Fe or Mn overlay very closely, but the active site has an appreciable RMSD. It is indeed known that reduction potential is a very sensitive quantity, altered dramatically even by residues in the second coordination sphere and weak H-bonds at the active site.[28,34] Additionally, long-range electrostatics play a significant role. This is well-known in modeling, where proper electrostatic embedding is required to reproduce the reduction potential.[35,36] Structural differences may also alter the electron conduction. Hence, there are a handful of unexplored possibilities to explain the Fe−Mn conundrum of RNR, but no conclusive explanation available to date.

Mn and Ni instead of Cu in superoxide dismutase (SOD) is another example of successful replacement of a redox metal (Figure 5). SODs are vital antioxidants in organisms that slow or prevent oxidative damage, inflammation, aging, and cancer.[37−39] They catalyze the disproportionation of the superoxide radical to yield O_2 and H_2O_2. SODs accomplish this through a two-step, ping-pong mechanism via a redox-active metal center, typically Fe or Mn, or a Cu/Zn pair, or with the unique case of Ni.[40] While our focus will be on Ni-SOD, we note a common link between Fe−Mn in RNRs and SODs. Mn can replace Fe in the role of redox metal in some SODs to avoid the deleterious effects of Fenton chemistry.[41] Interestingly as well, in certain bacteria, nickel levels enhance Ni-SOD expression while simultaneously repressing Fe-SOD expression, suggesting the evolution of Ni-SOD when Fe, or perhaps even Mn, concentrations were low.[42]

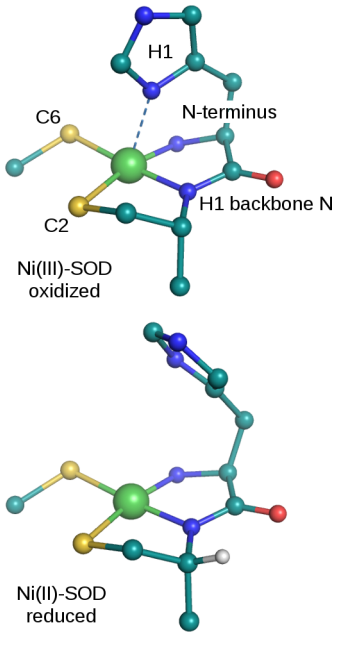

Figure 5. Two coordination modes of Ni in Ni-SOD.

Ni-SODs exist only in *Streptomyces* and cyanobacteria. Unlike all other metals utilized in SOD, Ni^{2+} does not catalyze superoxide dismutation in aqueous solution due to an improper redox potential (a calculated +2.26 V when the optimum reduction potential is 0.36 V).[43] The active site of Ni-SOD is strikingly different from those of the other SODs.[44] The Ni ion, coordinated by a "Ni-hook" motif,[45] has a square-pyramidal coordination geometry when Ni is in its oxidized form (Ni^{3+}). It has four equatorial ligands, two thiolates from Cys2 and Cys4, a deprotonated amide of the Cys2 backbone, and the N-terminal group of His1, and one axial amidazole from His1. Upon reduction, Ni^{2+} loses the His1 amidazole ligand and becomes square planar, making Ni-SOD the only observed SOD with a coordination number that changes as a function of metal oxidation state.[45,46] The flexible coordination geometry activates Ni and confers the proper redox potential needed to functional as a SOD.

In contrast to the specific coordination environment needed for Ni-SOD, **exchanging Mo^{4+} with W^{4+} in the enzyme DMSO reductase (DMSO-R)** for redox chemistry purposes sometimes yields an equally viable and active enzyme (Figure 6). Mo is used by all forms of life.[47,48] W is very close to Mo in coordination preferences, electronic properties, and ionic radii: 79, 75, and 73 pm for Mo, and 80, 76, and 74 pm for W, for the three oxidation states of VI, V, and VI, respectively. Organisms can be grouped according to those that prefer W, those that prefer Mo, and a significantly smaller group that can (to some extent) use both.[49] Most Mo-containing enzymes are mononuclear, and their activity involves transferring an oxygen atom to or from the substrate directly bound to the metal.[50] In Mo-DMSO-R, found in *Rhodabacter capsulatus*, the direct replacement with W is efficient and results in an active enzyme very close in structure to the Mo form (Figure 6).[1] W-DMSO-R was shown to be significantly more active than Mo-DMSO-R for the reduction of DMSO, but it does not oxidize DMS, unlike Mo-DMSO-R. The difference stems from the dissimilarity in the reduction potentials: W^{4+} is a stronger reductant and a weaker oxidant than Mo^{4+}. Redox behavior and the relative bioavailability of the metals (W is more bioavailable

dx.doi.org/10.1021/ar500227u | Acc. Chem. Res. 2014, 47, 3110−3117

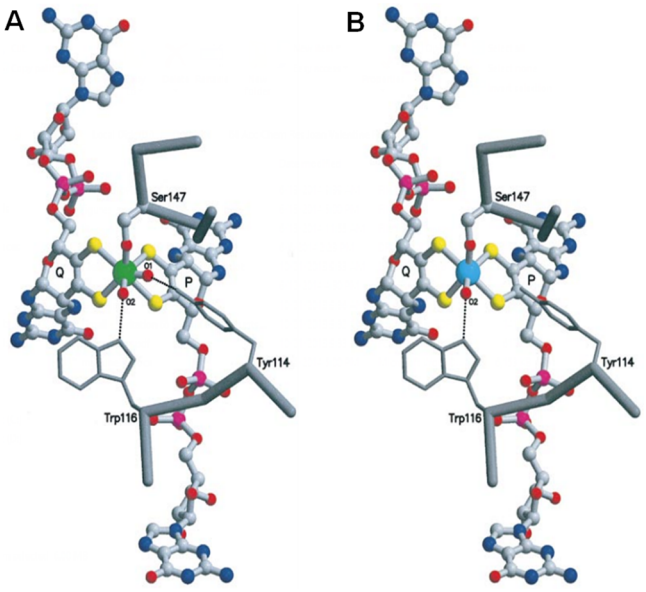

Figure 6. Structure of DMSO-R with (A) Mo, and (B) W. The RMSD between the two structures is 0.06 Å. Adapted with permission from ref 1. Copyright 2000 Elsevier.

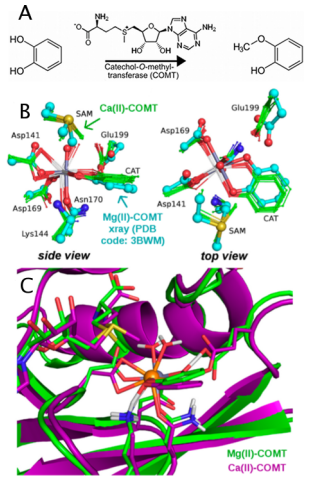

Figure 7. (A) Reaction catalyzed by COMT. (B) Comparing simulated Ca^{2+}-COMT structures (thin sticks, small spheres) with X-ray structure of Mg^{2+}-COMT (bold sticks, large spheres) reveals the shift of the methyl group on SAM with respected to catechol (CAT). (C) Representative simulation snapshots show overall structure adjustment when Mg^{2+} is replaced with Ca^{2+}.

than Mo)[51,52] are suggested to be major factors in determining whether an organism uses Mo or W in enzymes.

The rare organisms that can accommodate both Mo and W provide the link. The thermophilic methanogen *Methanobacterium wolfei* grows one of two isozymes determined by its environment.[53] In addition, growing *E. coli,* which usually uses Mo, on media containing W in the absence of Mo has been shown to generate an active enzyme with higher catalytic activity and increased thermal stability.[54]

CONSERVATIVE METAL REPLACEMENTS CAN DEACTIVATE

In contrast to dramatic redox exercises, simple template metal ions in enzymes are sometimes chosen perfectly for their purpose and cannot be replaced even by seemingly interchangeable metals. At first glance, the group 2 metals, Mg^{2+} and Ca^{2+}, are very similar electronically. Both metals interact with amino acids primarily by electrostatics, leaving the biggest difference between these two to be ionic radius, Mg^{2+} being smaller than Ca^{2+} and closer in size to common biologically used metals. Calcium's size, propensity for a higher coordination number,[55] and other related geometric strains[56] could be the sources of Mg^{2+} enzyme inhibition by Ca^{2+}. Interestingly, in some cases, enzymes preferentially bind Ca^{2+} over Mg^{2+}.[51,57,58] An example is the EF-hand motif, common to many calcium signaling and transport proteins, which undergoes conformational change to an inactive form when octahedrally coordinated Mg^{2+} is present.[57] These conformational changes can lead to a dramatic cascading effect, such as along the signaling transduction pathway.[58] We discuss catechol-*O*-methyltransferase to illustrate our point.

Catechol-*O*-methyltransferase (COMT) is a Mg^{2+} metalloenzyme involved in the regulation of catecholamine neurotransmitters in the brain and other organs (Figure 7A).[59,60] The enzyme catalyzes the transfer of the methyl group from the cofactor *S*-adenosyl-L-methionine (SAM) to catechol. The role of the metal in COMT is to bind and position the catechol substrate in the correct reactive orientation toward SAM for the methylation step.[61−63] Native Mg^{2+} in COMT can be replaced with Co^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, and Sn^{2+}.[64] However, metal replacement leads to varying changes in the activity and structure of the enzyme. The replacement of Mg^{2+} with Ca^{2+} leads to the inhibition of COMT. Fe^{2+} creates an only slightly weaker catalyst compared with Mg^{2+}, but Fe^{3+} is an inhibitor. We addressed the structure and function of these metal variants of COMT through an extensive computational experiment.[65] For Ca^{2+}-COMT, the reaction energetics are predicted to be unfavorable due to restructuring of the active site. The larger cation, Ca^{2+}, coordinates one additional ligand compared with Mg^{2+}, sits deeper in the binding cavity, and distorts the reacting parts of catechol and SAM out of alignment for methyl transfer (Figure 7B,C). Thus, inhibition is a purely geometric effect in this case. In contrast, the inhibitory effect of Fe^{3+} comes solely from the electronic properties of the metal, specifically its high electrophilicity.

METAL REPLACEMENT CAN ALSO REROUTE

Besides boosting or killing the native activity of enzymes, metal replacement can have much more sophisticated effects on enzyme function. One such example is **acireductone dioxygenases** (ARD). ARD is the only enzyme that utilizes the same protein scaffold to catalyze two different oxidation reactions, depending exclusively on whether Fe^{2+} or Ni^{2+} is bound.[66−68] Fe-dependent ARD′ oxidizes the substrate 1,2-dihydroxy-3-keto-5-(methylthio)pentene (acireductone) into two products: the α-keto acid precursor of methionine and formate. Fe-ARD′ is part of the methionine salvage pathway in cells.[68] Ni-dependent ARD instead oxidizes acireductone into three products, methylthiopropionate, CO, and formate, and provides a shunt out of the methionine salvage cycle.[68] Interestingly, the protein has micromolar affinity for both metals (K_d < 0.4 μM for Fe and K_d < 0.1 μM for Ni), readily allowing interconversion between the two. In contrast to other dioxygenases, such as non-heme iron dioxygenases that require the metal to activate the dioxygen for oxidation,[69,70] dioxygen binds the substrate directly.[71] The mechanistic difference between Fe and Ni for acireductone oxidation has been

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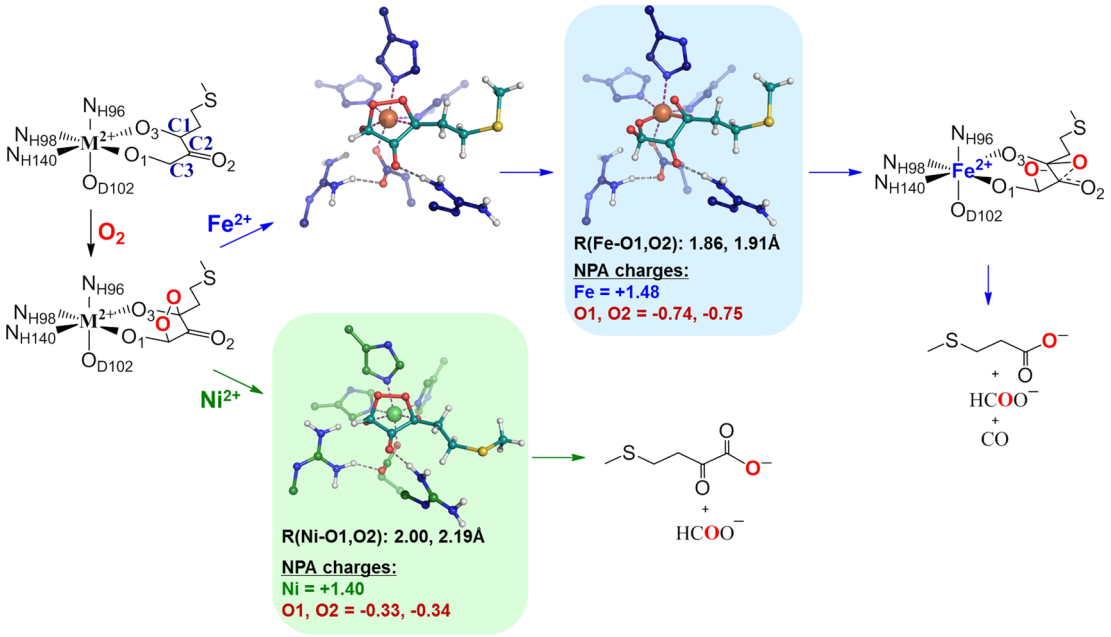

Figure 8. ARD/ARD′ reaction mechanisms, illustrating how the redox flexibility of Fe^{2+} allows for the electron transfer to the bound dioxygen and its dissociation, leading to a Fe-specific intermediate and a different reaction mechanism.

speculated to arise from coordination of the substrate to the metal center. However, recent experiments and computational studies challenge this view and propose a new explanation.[69,72,73] We found that the two metals bind the substrate in the same way, exposing the same sites to the nucleophilic attack by O_2 (Figure 8). Instead, an additional intermediate forms in the Fe-ARD′ mechanistic pathway that is not observed in Ni-ARD.[73] This extra intermediate is also found in biomimetic complexes mimicking ARD.[72] The ability of Fe but not Ni to stabilize an additional intermediate comes from the redox flexibility of Fe^{2+}, allowing for the flow of electrons from the residues to the bound O_2. The more electron-rich Ni^{2+} does not allow for this.[73]

Another interesting question is that of the functionality of intermediate Co-ARD. We showed computationally that for Co^{2+}-substituted ARD, both Fe and Ni routes are energetically accessible.[74] Experimentally, ARD has been observed to confer Ni-ARD activity with Mn^{2+} and Co^{2+}, whereas incorporation of Mg^{2+} confers Fe-ARD′ activity.[67,71]

CONCLUSIONS AND FUTURE OUTLOOK

By highlighting a variety of specific examples, this Account illustrates how the selection of a catalytic metal in the process of metalloenzyme evolution is based on factors other than optimizing catalysis alone. Some metal selections may be dictated by metal availability, as described in numerous examples of hydrolases. Alternate metal use, though surprising at first sight, is in fact meaningful from the standpoint of catalysis. Very similar metals, such as Mg^{2+} and Ca^{2+}, might have very different catalytic properties due to metal-induced large-scale structural effects. In other enzymes, such as the ARD/ARD′ system, the metal steers the catalyzed reaction in different ways due exclusively to the electronic effects. In some cases, radical metal substitution is tolerated by an enzyme, for example, redox active enzymes that adapt to use a metal with a very different reduction potential by tuning it through the nature of the ligands, strain, and other structural factors. For instance, some bacteria evolved to be able to survive in Fe^{2+} starvation by utilizing Mn^{2+} instead. SOD normally uses Cu or Fe to recycle superoxide, but some SODs can use Ni or Mn by forcing the needed reduction potential on these metals through their unusual ligand environments. These evolutionary moves may have been forced at some point by scarcity of the more commonly used metals or may have resulted from random mutations that enabled the organisms to expand their habitable environments. In summary, the discussion presented here further emphasizes that natural enzymes are not necessarily perfect catalysts but rather optimized within a broader evolutionary context. The enzyme design community aims to prepare maximally efficient quasi-natural enzymes for catalysis, with few examples of enzymes used by pharmaceutical manufacturers.[75] Metalloenzyme design specifically is unprecedented and provides a largely unexplored frontier for chemists, where roles of metals are extremely important but characterization can be problematic. These natural limitations provide an exciting opportunity for research into the improvement of metalloenzymes and their catalytic properties *in vitro*.

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Funding

Financial support of the NSF Graduate Fellowship No. 2011115747 (to C.E.V.), the DARPA Young Faculty Award N66001-11-1-4138, and Alfred P. Sloan Research Fellowship (to A.N.A.) are gratefully acknowledged.

Notes

The authors declare no competing financial interest.

Biographies

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