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Metal regulation of metabolism

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A broad range of biochemicals, from proteins to nucleic acids, function properly only when associated with a metal, usually a divalent cation. Not any divalent metal will do: these metals differ in their ionic radius, dissociation in water, ionization potential, and number of unpaired electrons in their outer shells, and so substituting one metal for another often changes substrate positioning, redox reactivities, and physiological performance, and thus may serve as a regulatory mechanism. For instance, exchanging manganese for magnesium in several chloroplast enzymes maintains plant carbon-nitrogen balance under rising atmospheric CO_2 concentrations. Here, we review this and a few other cases where association of proteins or nucleic acids with different metals control metabolism.

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Introduction

Biochemicals, including many proteins and nucleic acids, form complexes with divalent cation metals that allow the biochemicals to achieve bond angles and redox potentials that cannot be realized by polypeptides and nucleotides alone. Indeed, nearly half of all enzymes require metals in their active sites to perform catalysis [1]. Nucleic acids, in that they contain negatively charged phosphate groups at physiological pH, employ divalent metals as counter-ions [2].

The stability of divalent metals-biochemical complexes—in terms of the binding affinity of the aqueous metal for the biochemical ligand when it replaces water—increases from Mg^{2+} to Cu^{2+} according to the expanded Irving-Williams series [3–6]:

$$Mg^{2+} < Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}.$$

The exact location of Zn^{2+} in this series is uncertain, lying somewhere lower than Cu^{2+}, but higher than Co^{2+}.

Metals exist in several distinct pools within cells. For example, manganese in chloroplasts may belong to (*a*) 'free ionic' Mn^{2+}, (*b*) 'weakly bound' Mn^{2+} that is removable by EDTA, (*c*) 'strongly bound' Mn that is involved in water oxidation, and (*d*) 'very strongly bound' Mn that serves in the stacking of the chloroplast lamellae [7,8]. Activity of a metal is its 'effective concentration' in a solution containing a mixture of compounds; that is, the chemical potential of the metal depends on its activity in a real solution in the same manner that its chemical potential depends on concentration in an ideal solution. Only 'free ionic' and 'weakly bound' metals contribute significantly to the activity of a metal.

Cells regulate activities of metals (a_{Me}'s) in reverse order to that of affinities for them [9,10] (Table 1):

$$a_{Mg}{}^{2+} > a_{Mn}{}^{2+} > a_{Fe}{}^{2+} > a_{Co}{}^{2+} > a_{Ni}{}^{2+} > a_{Zn}{}^{2+} > a_{Cu}{}^{2+}.$$

Cellular activities range from Mg^{2+} whose the activity is high enough to be considered a macronutrient, to Cu^{2+} whose activity averages less than one atom per cell [11].

With affinities and activities trending in opposite directions, dissociation constants (K_d's) become roughly equal to the activities of the metals, and the interactions between biochemicals and metals become relatively fast and loose. This is because if a biochemical B associates with a metal Me^{2+} on a one-to-one basis (i.e. $B{\cdot}Me \leftrightarrow B + Me^{2+}$), the dissociation constant of the reaction is defined by

$$K_d = \frac{a_B \cdot a_{Me^{2+}}}{a_{B \cdot Me}}.$$

When $a_{Me}{}^{2+} = K_d$, then $a_B = a_{B \cdot Me}$; in words, the activities of the unassociated and metal-associated biochemical are the same when the Me^{2+} activity equals K_d. Under such conditions, the metal relatively rapidly associates with and disassociates from the biochemical [12]. This presents a major problem: techniques employed for isolating a specific biochemical often displace the associated metal, and so determining which metal is associated *in vivo* becomes difficult.

Binding sites within proteins and nucleic acids may physically accommodate different metals because these metals not only have the same charge, but also exhibit similar ionic radii in aqueous solutions (Table 1). Displacing one metal with another, however, may change the chemistry of the biochemical including the pK_a of the metal/H_2O complex (Table 1), ionization potential

Table 1

Some properties of divalent cation metals. "Ionic radius, low spin" designates that the metal ion has as many of its electrons paired as possible. "pK_a metal/H_2O complex" designates the reaction $[Me(H_2O)_6]^{2+} \rightarrow [Me(H_2O)_5OH]^{+} + H^{+}$. "Ionization potential" designates the energy required to remove electrons from the metal, Me to Me^{2+}, and reflects the Lewis base strength

Metal	Mg^{2+}	Mn^{2+}	Fe^{2+}	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}	Reference
Cytosolic activity (mM)	0.8	0.01	0.005	10^{-6}	10^{-6}	10^{-15}	10^{-9}	[9]
Ionic radius (Å), low spin	0.65	0.67	0.61	0.65	0.69	0.69	0.71	[28]
pK_a metal/H_2O complex	11.2	11.0	9.5	9.7	9.9	8	9.9	[38]
Ionization potential (eV)	15.04	15.64	16.18	17.06	18.17	20.29	17.96	[1,39]

(Table 1), redox reactivity, ligand binding, or binding geometry. Such changes can render a protein or nucleic acid nonfunctional leading to physiological disorders including neurodegenerative diseases [13].

Beneficial metal substitutions

Not all metal substitutions are deleterious. For example, Mn^{2+} and Fe^{2+} bind relatively weakly to proteins or nucleic acids and prefer similar coordination environments; therefore, proteins or nucleic acids have difficulty in distinguishing between these metals on the basis of structure alone [14•]. Yet the redox chemistry of the two metals are highly disparate, and aerobic organisms may substitute Mn^{2+} for Fe^{2+} in proteins to avoid oxidative damage and iron deficiencies [14•]. In *Escherichia coli*, several enzymes that use Fe^{2+} to bind substrate and to stabilize electrostatically an oxyanionic intermediate suffer damage when exposed to oxidative stress agents such as H_2O_2 and $O_2^{\bullet-}$ that the bacterium may normally encounter [15]. Under oxidative stress, *E. coli* activates a transcriptional regulator that upregulates Mn^{2+} import and Fe^{2+} sequestration, and thus, Mn^{2+} replaces of Fe^{2+} within these enzymes and they are able to sustain near normal activity [15].

Another example of a beneficial metal substitution is the enzyme acireductone dioxygenase (ARD), which is part of the methionine salvage pathway in the bacterium *Klebsiella oxytoca* in which it catalyzes two different reactions depending on whether Fe^{2+} or Ni^{2+} occupies the active site [16]. When associated with Fe^{2+}, ARD catalyzes the reaction in which acireductone and dioxygen generate formate and the ketoacid precursor of methionine, 2-keto-4-methylthiobutyrate (KMTB) (Figure 1a). When associated with Ni^{2+}, the enzyme catalyzes the reaction in which the same substrates generate methylthiopropionate (MTB), carbon monoxide, and formate (Figure 1a). ARD is promiscuous and also forms associations with Co^{2+} or Mn^{2+} that promote a Ni^{2+}-like reaction or with Mg^{2+} that promote a low level Fe^{2+}-like reaction [17]. How the dual chemistry of the ARD enzyme serves to regulate the methionine salvage pathway is still unknown [18•].

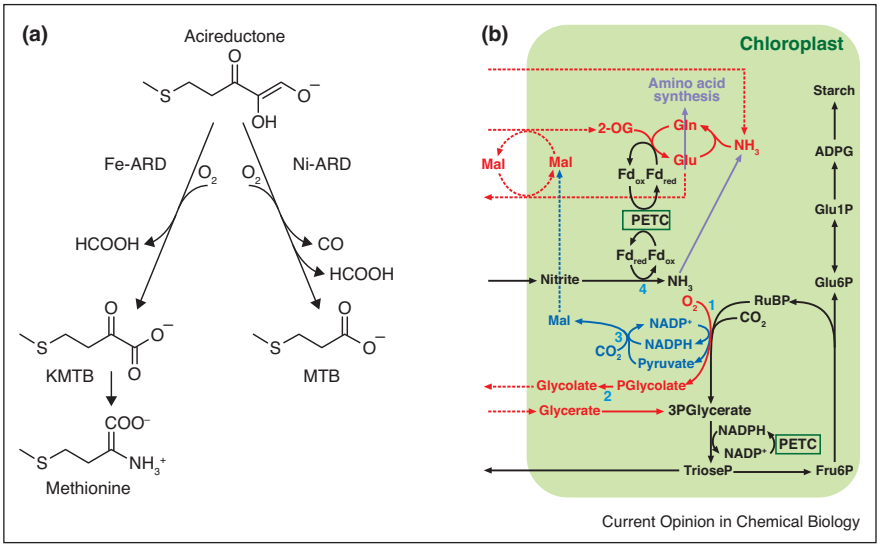
Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), the most prevalent protein on the planet [19], provides another example of 'one protein, two enzymes'. Rubisco catalyzes either carboxylation of the substrate RuBP to initiate the C_3 carbon fixation pathway or oxidation of RuBP to initiate the photorespiratory pathway (Figure 1b). The balance between the carboxylation and oxygenation reactions depends on several factors, but the one that has been ignored is the extent to which Rubisco associates with either Mg^{2+} or Mn^{2+}. When Rubisco associates with Mn^{2+}, carboxylation and oxidation proceed at similar rates (Table 2) [20], the oxygenation produces singlet oxygen [21,22], and the Mn^{2+} transfers an electron with every oxidation [22]. When Rubisco associates with Mg^{2+}, carboxylation accelerates and proceeds about four times faster than oxidation (Table 2), but no electrons are transferred [23].

Carboxylation of RuBP when Rubisco associates with Mg^{2+} results in a reaction enthalpy change ($\Delta_r H$) of $-21\,kJ\ mol^{-1}$, whereas oxygenation of RuBP when Rubisco associates with Mn^{2+} results in a $\Delta_r H$ of $-319\,kJ\ mol^{-1}$, more than 15 times greater [24]. The prevailing view is that the initial reaction of the photorespiratory pathway is $RuBP + O_2 + H_2O \rightarrow$ glycolate + 3-phosphoglycerate + P_i and that the energy released during RuBP oxidation is dissipated as waste heat [24].

We proposed an alternative pathway [25••] in which the electrons transferred by Mn^{2+} during the oxidation of RuBP reduce $NADP^+$ to NADPH, and so the initial reaction of photorespiration becomes $RuBP + NADP^+ + H^+ + O_2 + H_2O \rightarrow$ pyruvate + glycolate- + NADPH + $2P_i$. Next, Mn^{2+}-malic enzyme catalyzes the reaction pyruvate + CO_2 + NADPH $\rightarrow$ malate + $NADP^+$. Together, the net result becomes $RuBP + O_2 + CO_2 + H_2O \rightarrow$ glycolate + malate + $2P_i$ (Figure 1b). The additional malate generated by this alternative pathway empowers many energy-intensive biochemical reactions such as those involved in nitrate assimilation. Thus, photorespiration may be much more energy efficient than most researchers have presumed [25••].

We recently quantified Mg^{2+} and Mn^{2+} activities in isolated tobacco chloroplasts [20]. Mg^{2+} was more active than Mn^{2+} ($a_{Mg^{2+}} \approx 3$ mM versus $a_{Mn^{2+}} \approx 20\,\mu M$). The activities in the chloroplasts were roughly proportional to the concentrations in the medium, indicating that regulation of metal activities occurred at the cellular level

Figure 1



Beneficial metal substitutions. **(a)** The reactions catalyzed by Fe-acireductone dioxygenase and Ni-acireductone dioxygenase that are part of the Methione Salvage Pathway in the bacterium *K. oxytoca*. **(b)** A proposed photorespiratory pathway within a chloroplast. The solid red lines represent reactions of the photorespiratory pathway, the solid blue lines represent reactions of the proposed alternative photorespiratory pathway favored by the association of Rubisco with Mn^{2+}, the solid purple lines represent reactions of amino acid synthesis, and the dotted lines represent associated transport processes. Numbered reactions are catalyzed by the following enzymes: 1. Rubisco, 2. Phosphoglycolate phosphatase, 3. Malic enzyme, and 4. Nitrite reductase. PETC designates photosynthetic electron transport chain [25••].

perhaps via regulation of plasma membrane transport. We also assessed the thermodynamics of metal binding to Rubisco purified from tobacco [20]. The Rubisco had a higher dissociation constant (K_d) for Mg^{2+} than Mn^{2+} (1.7 mM for Mg^{2+} versus 14 μM for Mn^{2+}), and so the K_d's for each metal was similar in magnitude to the activity of each in the chloroplast. Thus, Rubisco associates almost equally with both metals and rapidly exchanges one metal for the other.

The ratio of Mg^{2+} contents to Mn^{2+} contents in plant leaves decreases under elevated atmospheric CO_2 [26] and when plants receive nitrate rather than ammonium as a nitrogen source [20]. This suggests that plants regulate Mg^{2+} and Mn^{2+} activities in cells to mitigate changes in photorespiration and the concomitant changes in nitrate assimilation [20]. Through this regulation, plants avoid detrimental shifts in nitrogen/carbon balance during diurnal fluctuations in atmospheric CO_2 concentrations.

Table 2

Maximum velocity (μmol min^{-1} mg^{-1} protein) of carboxylation (V_c) or oxygenation (V_o) for Rubisco purified from different sources when it was associated with manganese or magnesium (mean ± SE, n = 6). [20]

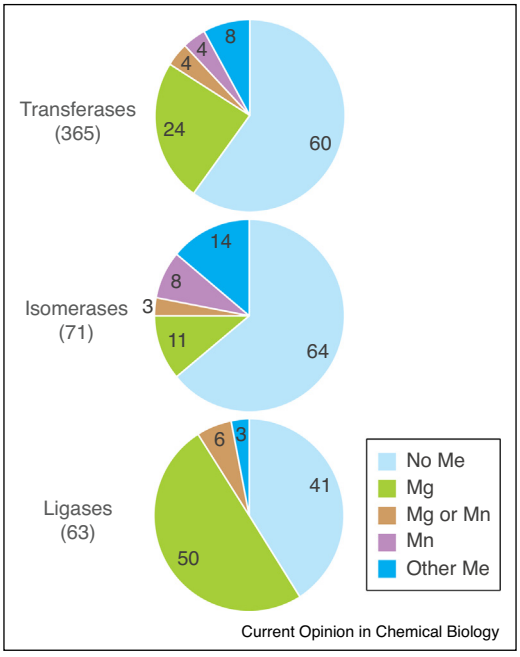
Metal	V_c	V_o
Mn^{2+}	0.41 ± 0.17	0.30 ± 0.07
Mg^{2+}	1.60 ± 0.33	0.42 ± 0.11

Mg^{2+} versus Mn^{2+} as cofactors

Mg^{2+} and Mn^{2+} are similar in many ways: their pK_a's for disassociation in water, their ionization potentials, and their ionic radii in a low spin state, where the metals have as many electrons paired as possible, are nearly identical (Table 1). They form similar binding geometries with similar ligands [27]. Perhaps because of these similarities, Mg^{2+} and Mn^{2+} often substitute for one another in the metal binding site of enzymes. Of transferases, isomerases, and ligases that associate with metals, 61% are fully functional when associated with Mg^{2+}, 10% with Mn^{2+}, but 10% with either Mg^{2+} or Mn^{2+} (Figure 2). The three other classes of enzymes (oxidoreductases, hydrolases, and lyases) do not associate with Mg^{2+} interchangeably for Mn^{2+}.

Mg^{2+}, however, has only paired electrons in it outer shell and does not participate in redox reactions under physiological conditions, whereas Mn^{2+} has up to five unpaired electrons in its outer shell and readily participates in redox reactions. A shift from a low spin state to a high spin state (from a minimum to maximum number of unpaired electrons), does not influence the ionic radius of Mg^{2+}, which remains at 0.65 Å, whereas that of Mn^{2+} increases from 0.67 to 0.82 Å [28]. The inner hydration shell Mg^{2+} is regular, whereby its octahedral shape is precisely maintained at ion–water distances of 2.08 Å [29]. The hydration geometry of Mn^{2+} is similar to Mg^{2+}, but its ion–water distances are 2.3 Å and its octahedral

Figure 2



Proportion (%) of transferases, isomerases, or ligases that have in their catalytic site either no metal, Mg^{2+}, Mg^{2+} and Mn^{2+}, Mn^{2+}, or another metal. Oxidoreductases, hydrolases, and lyases did not associate with Mg^{2+} interchangeably for Mn^{2+}. These are enzymes with structures deposited in the Protein Data Bank. In parantheses are the total number of enzymes with known structures. For example, 60% of the 365 transferases with known structures are not associated with a metal, whereas 24% are associated with Mg^{2+}, 4% with either Mg^{2+} or Mn^{2+}, 4% with Mn^{2+}, and 8% with another metal [1].

symmetry is often distorted [29]. Such differences in the electron transfers and hydration geometry are likely to influence the function of a protein when it associates with one metal versus the other, but detailed information is lacking.

The most common ligand for both Mg^{2+} and Mn^{2+} is oxygen, although this is more dominant for Mg^{2+} in which 77% of the bonds for Mg^{2+}in the Cambridge Structural Database are with oxygen, whereas only 61% of the bonds for Mn^{2+} are with oxygen [27]. Reactions in which nitrogen-containing ligands are transferred from Mg^{2+} to Mn^{2+} ions, are all exothermic ($-10.8 < \Delta H^{\circ}_{298} < -2.9\,\text{kcal mol}^{-1}$). This confirms that Mg^{2+} is less accepting of nitrogen atoms as a ligand than is Mn^{2+}. The value of ΔH°_{298} for the transfer reaction $Mg^{2+} + Mn[H_2O]_6{}^{2+} \rightarrow Mg[H_2O]_6{}^{2+} + Mn^{2+}$ is significantly exothermic $-19.4\,\text{kcal mol}^{-1}$, indicating an energetic preference of water for Mg^{2+} [27]. The ligand preferences of Mg^{2+} or Mn^{2+} should influence the protein with which the metal associates, but again little information is available.

Mg^{2+} and Mn^{2+} associate not only with proteins, but also with nucleotides and nucleic acids. Perhaps best studied is the complex between Mg^{2+} or Mn^{2+} and phosphate groups of ATP. The metals are critical for the interactions between the ATP and proteins. The log of the stability constant between ATP and Mg^{2+} and $Mn^{2+}$is 4.3 and 5.0, respectively [2]. Oligonucleotides interact with Mg^{2+} primarily through its inner-sphere hydration waters, but they also interact directly with Mg^{2+} and Mn^{2+} through ion–N7 (guanine) and ion–phosphate groups [29]. RNAs, because of their high negative charge, are associated with large numbers of metal ions for charge compensation. Mg^{2+} seems to be the natural cofactor in that maximum catalytic rates are achieved in its presence [2], and providing Mg^{2+} at biologically realistic activities promotes RNA stability and catalysis [30•]. By contrast, some small catalytic RNAs like the hammerhead ribozyme, exhibit maximum rates in the presence of Mn^{2+}.

Conclusions: metal regulation of biochemicals

Despite the profound effects of metals on the structure and function of proteins or nucleic acids and the wealth of data on the physical chemistry of metallo-biochemical complexes, information about the influence of metal associations on metabolic regulation remains sparse. This derives in part from the lack of appropriate methodologies. Fortunately, new methods are addressing this issue.

Only 'free ionic' or 'weakly bound' metals participate in cellular metabolism, but standard methods for quantifying metals such as inductively-coupled plasma atomic emission spectroscopy do not differentiate among the 'free ionic', 'weakly bound', 'strongly bound', and 'very strongly bound' pools. Fluorescent dyes, which provide estimates of the metabolically active metal pools [31], have elucidated the role of calcium as a secondary cellular messenger [32] and, more recently, the importance of cellular Mg^{2+} to Mn^{2+} ratios in controlling plant carbon/nitrogen balance [20].

Purifying proteins or nucleic acids without displacing associated metals, as mentioned above, is still challenging. A variety of more gentle methods for purification such as size-exclusion chromatography and capillary electrophoresis are now being used [33,34•,35]. Also techniques for imaging metals in tissues with higher spatial resolution such as matrix assisted laser desorption/ionization in combination with inductively-coupled plasma mass spectrometry are becoming more common [36,37]. Once data are available on what, where, and when metal-biochemical associations occur, metabolic metallomics will mature from a discipline with a few anecdotes to one integrated into chemical biology.

Conflicts of interest statement

Nothing declared.

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