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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