Determinants of Fe$^{2+}$ over M$^{2+}$ ($M = Mg, Mn, Zn$) Selectivity in Non-Heme Iron Proteins

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ABSTRACT: Iron cations are indispensable players in a number of vital biological processes such as respiration, cell division, nitrogen fixation, oxygen transport, nucleotide synthesis, oxidant protection, O$_2$ activation in the metabolism of various organic substrates, gene regulation, and protein structure stabilization. The basic mechanisms and factors governing the competition between Fe$^{2+}$ and other metal species from the cellular fluids such as Mg$^{2+}$, Mn$^{2+}$, and Zn$^{2+}$ are, however, not well understood, and several outstanding questions remain. (i) How does the Fe$^{2+}$ binding site select the “right” cation and protect itself from attacks by other biogenic cations present in the surrounding milieu? (ii) Do the iron binding sites employ different selectivity strategies toward metal cations possessing different ligand affinities and cytosolic concentrations? (iii) What are the key determinants of metal selectivity in Fe$^{2+}$ proteins? In this study, by employing density functional theory calculations combined with polarizable continuum model computations, we endeavor to address these questions by evaluating the thermodynamic outcome of the competition between Fe$^{2+}$ and Mg$^{2+}$/Mn$^{2+}$/Zn$^{2+}$ in model non-heme mononuclear metal binding sites of various compositions and charge states. The present calculations, which are in line with available experimental data, shed light on the mechanism of Fe$^{2+}$–Mg$^{2+}$/Mn$^{2+}$/Zn$^{2+}$ competition in non-heme iron proteins and disclose the key factors governing the process.

INTRODUCTION

Over the course of 2–3 billion years of cell evolution, about two dozen metal species have been selected and bestowed biological function ranging from enzyme catalysis to nucleic acid and protein structure stabilization, signal transduction, muscle contraction, hormone secretion, taste and pain sensation, blood coagulation, respiration, and photosynthesis. However, the simplest, yet most versatile, participants in biochemical processes with a multitude of characteristic properties such as positive charge, flexible coordination sphere, specific ligand affinity and Lewis acidity, varying valence state and spin configuration, and specific mobility/diffusivity. The most abundant biogenic metal cations, chosen on the basis of their physicochemical properties and bioavailability, are Na$^+$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, Zn$^{2+}$, Mn$^{2+}$, Fe$^{2+/3+}$, Co$^{2+/3+}$, Ni$^{2+}$, and Cu$^{1+/2+}$.

Iron, a redox-active element with oxidation state alternating between +2 and +3 (and sometimes +4), is an indispensable player in a number of vital biological processes such as respiration, cell division, nitrogen fixation, oxygen transport, nucleotide synthesis, oxidant protection, O$_2$ activation in the metabolism of various organic substrates, gene regulation, and protein structure stabilization. Depending on the structure of the metal binding site, iron proteins can be classified into several groups: (1) heme proteins where the iron cation is coordinated to a heme porphyrin ring, which, on its side, is bound covalently or noncovalently to the host protein; (2) iron–sulfur cluster proteins comprising [2Fe–2S], [3Fe–4S], and [4Fe–4S] clusters, embedded in the protein structure and coordinated to the protein, most often by Cys ligation to the cluster’s iron atoms; (3) mono- and polynuclear non-heme proteins possessing iron binding sites lined with amino acid residues that donate the host protein.

The composition of metal binding sites in representative mononuclear non-heme Fe$^{2+}$ proteins is presented in Table 1. The data collected demonstrate that the preferred binding partners of Fe$^{2+}$ are His and Asp/Glu side chains. The latter usually coordinate to the metal in a monodentate fashion. The typical Fe$^{2+}$ binding site configuration is His$_5$(Asp/Glu)$_1$, denoted the “2-His-1-carboxylate facial triad motif.” It is a signature motif for a large group of iron dioxygenases, hydrolases, and synthases. Other combinations between His and acidic residues exist as well: His$_5$(Asp/Glu)$_2$, His$_5$(Asp/Glu)$^-$, and His$_5$(Asp$^-$/Glu$^-$). The coordination number of Fe$^{2+}$ varies between 5 and 6 with water or substrate molecules complementing the coordination sphere.

Inside the cell, Fe$^{2+}$ has to compete for protein binding sites with other biogenic metal species, such as Mg$^{2+}$, Mn$^{2+}$, and Zn$^{2+}$. Although these cations have the same charge and similar ionic radii ($R_{Fe^{2+}} = 0.78$ Å, $R_{Mg^{2+}} = 0.72$ Å, $R_{Zn^{2+}} = 0.74$ Å, $R_{Mn^{2+}} = 0.83$ Å for hexacoordinated ions), they possess different ligand affinities, as reflected in the Irving–Williams series:

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

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Table 1. Composition of Fe²⁺ Binding Sites in Representative Mononuclear Non-Heme Proteins

| protein                                      | reference/ PDB entry | Fe²⁺-binding ligands³⁹ |
|----------------------------------------------|-----------------------|------------------------|
| 2,3-dihydroxyphenyl dioxygenase              | 1K3W                  | H114, H209, E260, 2H₂O |
| HPP dioxygenase                              | 1CJX                  | H161, H240, E322, Sub⁴⁰ |
| homogentisate dioxygenase                    | 1EY2                  | H335, E341³⁹, H371, 2H₂O|
| human factor inhibiting HIF                  | 1MZF                  | H199, D201, H279, Sub⁴¹ |
| human phenylalanine dehydroxylase            | 1J8U                  | H285, H290, E330, 3H₂O |
| homoprotocatechuate 2,3-dioxygenase          | 15                    | H155, H214, E267, Sub⁴¹ |
| carbonan synthase                            | 11                    | H101, D103, H251, Sub⁴¹ |
| clavamin synthase                            | 1DS1                  | H144, E146, H279, H₂O,  |
| deacetoxycephalosporin C synthase            | 18                    | H183, D185, H243, H₂O,  |
| isopenicillin N synthase                     | 2VI                   | H214, D216, H270, H₂O,  |
| extradiol dioxygenase                        | 19                    | H146, H210, E260, 2H₂O |
| TaulD                                        | 1OS7                  | H99, D101, H255, Sub⁴¹ |
| alkylsulfatase Atsk                          | 1O1I                  | H108, D110, H264, H₂O,  |
| histone deacetylase 8                        | 3MZ6                  | D178, H180, D267, 2H₂O, |
| d-ribulose 5-phosphate 3-epimerase           | 3OVP                  | H35, D37, H70, D175, 2H₂O|
| lipoxynase                                   | 1F8N                  | H499, H504, H690, N694, |
| 5-methylcytosine deaminase                   | 4R7W                  | H56, H58, H209, D308,  |
| "Sub" stands for a substrate molecule. Residues/substrates with the superscript "bi" coordinate to the metal ion in a bidentate fashion. Monodentate binding is observed for other residues (with no superscript). The superscript "ox" signifies an end carboxylate group. Water molecules within <3 Å of Fe²⁺ are assumed to be bound to the metal cation. |

Magnesium and manganese ions, located at the far left-hand side of the series, have weaker ligand affinities than Fe²⁺. Zinc cations, on the other side, exhibit much higher ligand coordinating strength than Fe²⁺ and form, as a rule, more stable complexes. Furthermore, these metal contenders are present in different free cytoscopic concentrations ranging from millimolar for Mg²⁺ to micromolar for Fe²⁺ and Mn²⁺ and picomolar/femtomolar for Zn²⁺. Note that the respective concentrations in the resting cell generally correlate inversely to the thermodynamic outcome of the competition between Fe²⁺ and Mg²⁺/Mn²⁺/Zn²⁺ in model non-heme mononuclear metal binding sites of various compositions and charge states (see Methods). Density functional theory (DFT) calculations in combination with polarizable continuum model (PCM) computations are employed. The Fe²⁺ binding site ligand selectivity can be expressed in terms of the free energy, ΔG°, for replacing the “native” Fe²⁺ bound to the protein by its rival cation, M²⁺ (M = Mg, Mn, Zn):

\[
[M²⁺-aq] + [Fe²⁺-protein] \rightarrow [M²⁺-protein] + [Fe²⁺-aq]
\]

(1)

In eq 1, [Fe²⁺/M²⁺-protein] and [Fe²⁺/M²⁺-aq] represent the metal ion bound to protein ligands and unbound in the vicinity of the binding site, respectively. The binding cavity is characterized with an effective dielectric constant, ε, varying from ~4 for buried binding sites to ~30 for solvent-accessible binding pockets. A positive ΔG° implies a Fe²⁺-selective site, whereas a negative value implies a M²⁺-selective site. Our aim is to obtain reliable trends in the free energy changes with varying parameters of the system such as the structure, composition, overall charge, and solvent exposure of the metal binding site rather than reproduce the absolute ion exchange free energies in these metal centers. Notably, trends in the free energies computed using this approach have been found to be consistent with experimental observations in previous works.³³⁻³⁵

Methods

Models Used. The side chains of Asp/ Glu⁻ and His were modeled as acetate (CH₃COO⁻) and imidazole (C₅H₄N₅⁻), respectively. The metal cations under study (Fe³⁺, Mg²⁺, Mn²⁺, and Zn²⁺) are usually hexahydrated in aqueous solution.³⁴,³⁵ Hence, their aqua complexes were modeled as [M(H₂O)₆]²⁺ (M = Fe, Mg, Mn, Zn). In protein binding sites, Fe²⁺ is typically coordinated to five or six ligands (²⁻⁷) (see Table 1 as well). Accordingly, the respective five- and six-coordinated complexes were modeled as \{[Fe(H₂O)₅](L)₅⁻\}²⁻ and \{Fe(H₂O)₆(L)₄⁻\}³⁻, where L = CH₃COO⁻, imidazole, N₂⁻, and p = 0, 1. The structure of Fe²⁺ complexes was used as a starting point for geometry optimization of other metal complexes. The overall structure of the resulting Mg²⁺, Mn²⁺, and Zn²⁺ complexes did not change in the course of geometry optimization; thus, the coordination numbers of 5 and 6 were retained in the respective optimized structures. High-sin configurations for Fe²⁺ (quintuplet) and Mn²⁺ (sextuplet) were considered in line with the experimental and theoretical findings.³⁶,³⁷

DFT/PCM Calculations. The M06-2X method³⁸ in conjunction with the 6-311+G(dp) basis set was employed to optimize the geometry of each metal complex in both the gas phase and condensed media and compute the electronic energies, Eₑ, using the Gaussian 09 suite of programs.³⁹ Catalytic metal binding sites are located in cavities/crevices of the protein structure whose dielectric properties differ from that of the bulk water⁴⁰ and are comparable to those of the low-polarity solvents.⁴¹ Thus, solution-phase computations were conducted in solvents mimicking the dielectric properties of buried and solvent-accessible binding sites: diethyl ether (ε = 4) and propanonitrile (ε = 29), respectively. Frequency calculations for each optimized structure were performed at the same M06-2X/6-311+G(dp) level of theory. No imaginary frequency was found for any of the optimized structures. The frequencies were scaled by an empirical factor of 0.983⁴² and used to compute the thermal energies, including zero-point energy, and entropies. The electronic energies in solution were corrected by performing single-point calculations on the respective fully optimized structures employing the SMD solvation model.⁴³ The differences ΔEₑₙ, ΔEₑₙ°, and ΔS° between the products and reactants in eq 1 were used to calculate the metal exchange free energy at T = 298.15 K according to

\[
ΔG° = ΔEₑₙ° + ΔEₑₙ + ΔS°
\]

(2)

The basis set superposition error for this type of exchange reaction (eq 1) had been shown to be negligible⁴⁴ and was thus not considered in the present calculations. The theoretical method and calculation protocol used were validated with respect to available experimental data and proven to be reliable, as they reproduced correctly the geometries of Mg³⁺, Fe³⁺,
Mn²⁺, and Zn²⁺ representative structures as well as the free energies of Mg²⁺/Mn²⁺/Zn²⁺ → Fe²⁺ exchange in acetate, imidazole, and glycine complexes (Table 2).

Table 2. Comparison between Computed and Experimental Averaged Metal–Oxygen Bond Distances and Metal-Exchange Free Energies, ΔG, in M²⁺ (M = Mg, Fe, Mn, Zn) Complexes

| complex or reaction | expbl | calcld |
|---------------------|-------|--------|
| [Mg(H₂O)₆]²⁺        | 2.07 ± 0.03⁷ | 2.06   |
| [Zn(H₂O)₆]²⁺        | 2.08 ± 0.03³ | 2.11   |
| [Fe(H₂O)₆]²⁺        | 2.12/2.13²⁷ | 2.15   |
| [Mn(H₂O)₅(acetate)]²⁺ | 2.17–2.22² | 2.19   |
| [Zn(H₂O)₅(imidazole)]²⁺ | 0.8⁵ | 2.6    |
| [Mn(H₂O)₅(imidazole)]²⁺ | −1.8⁵ | −2.4   |
| [Zn(H₂O)₅(glycine)]²⁺ | −0.8⁴ | −2.8   |
| [Mg(H₂O)₅(glycine)]²⁺ | 0.3⁴ | 2.6    |
| [Mg(H₂O)₄(glycine)]²⁺ | 0.3⁴ | 3.4    |
| [Zn(H₂O)₄(glycine)]²⁺ | 0.3⁴ | 4.8    |

“From ref 45. From ref 46. From ref 43. Evaluated from the stability constants in water solution provided by ref 47. Evaluated from the stability constants in water solution provided by ref 48.

RESULTS

2-His-1-Carboxylate Facial Triad Motif. A large group of mononuclear non-heme Fe²⁺ proteins possesses metal binding sites lined with two neutral His side chains and an anionic Asp²⁻/Glu⁻ residue (“2-His-1-carboxylate facial triad motif”).⁴⁰–⁵¹ Since the coordination number of Fe²⁺ in these complexes is 5 or 6 (Table 1), we modeled both penta- and hexacoordinated Fe²⁺ binding sites comprising two imidazoles (model for His side chain), one acetate (model for Asp²⁻/Glu⁻ residues), and three water molecules (Figure 1). The three water ligands are directly bound to the metal cation in the hexacoordinated complex (Figure 1b), whereas one of them is transferred to the second coordination shell in the pentacoordinated counterpart (Figure 1a).

Figure 1 depicts the structures of penta- and hexacoordinated Fe²⁺ complexes along with the free energies of metal exchange Mg²⁺/Mn²⁺/Zn²⁺ → Fe²⁺ in the gas phase, ΔG, buried protein binding site, ΔG⁴, and solvent-accessible metal center, ΔG⁹⁹. The results obtained reveal the following trends. (i) Mg²⁺ and Mn²⁺ cannot successfully compete with Fe²⁺ for the binding site, as evidenced by the positive free energies of metal substitution in both the gas phase and condensed media. This finding is not surprising in view of the weaker ligand affinities of Mg²⁺ and Mn²⁺ to Fe²⁺ cations relative to those of the Fe²⁺ cation (see Introduction). However, Mn²⁺, being closer in physicochemical properties to Fe²⁺ than Mg²⁺ to Fe²⁺, is a more potent iron contender than Mg²⁺ (less positive free energies for the Mn²⁺ → Fe²⁺ exchange than for the Mg²⁺ → Fe²⁺ substitution). (ii) The Fe²⁺ binding sites are not well protected against attacks by the rival Zn²⁺ cations, which form more stable complexes and are able to displace Fe²⁺ from the respective metal centers (negative ΔG values for the Zn²⁺ → Fe²⁺ exchange in Figure 1). (iii) Solvation does not appear to be a major determinant of the Fe²⁺/Mn²⁺ and Fe²⁺/Zn²⁺ selectivity in these systems, as it only slightly affects the free energies of metal substitution and does not alter the trends observed in the gas phase. The effect is stronger for the Fe²⁺/Mg²⁺ competition in pentacoordinated metal centers, where the increased solvent exposure of the binding site favors the Fe²⁺/Mg²⁺ selectivity. (iv) Reducing the metal coordination number from 6 to 5 has a dual effect on the binding site selectivity: On one hand, it increases the Fe²⁺/Mg²⁺ selectivity, as the pentacoordinated Mg²⁺ complex is less favored than its hexacoordinated counterpart (resulting in higher positive numbers for the Fe²⁺/Mg²⁺ competition in the pentacoordinated complexes in Figure 1a than in the hexacoordinated structures in Figure 1b). On the other hand, it renders binding pockets less selective for Fe²⁺ over Zn²⁺, since the Zn²⁺ binding is favorably affected by reducing the metal coordination number (more negative ΔG values for the Fe²⁺/Zn²⁺ competition in the pentacoordinated complexes in Figure 1a than for hexacoordinated constructs in Figure 1b).

The above findings apply to flexible Fe²⁺ binding sites that, in response to the specific physicochemical requirements of the guest noncognate metal, can readjust their structures upon replacing Fe²⁺ with Mg²⁺/Mn²⁺/Zn²⁺, thus securing optimal accommodation for the incoming contender. Would, and if so, to what extent, the above trends change if the Fe²⁺ binding site were rigid and did not allow for any structural rearrangements of the protein ligands upon metal substitution? In addressing this question, we replaced Fe²⁺ with Mg²⁺, Mn²⁺, or Zn²⁺ in the Fe²⁺-optimized Fe-3wat-2imi-1ace construct and performed partial geometry optimization of the resulting metal complex,
keeping the positions of all protein ligands frozen (thus preserving the original cavity size unchanged) but allowing water molecules to reoptimize their positions. The electronic energies of the partially optimized structures were used in computing the Mg$^{2+}$/Mn$^{2+}$/Zn$^{2+} \rightarrow$ Fe$^{2+}$ substitution free energies (numbers in parentheses in Figure 1b), which provide an upper limit of the rigidity effect on the metal selectivity in such inflexible non-heme binding sites. The results imply that rigidifying the metal binding site enhances the Fe$^{2+}$ competitiveness over its non-native contenders (higher $\Delta G^{\text{ex}}$ by $\approx$2 kcal/mol), although it does not change the trends found for flexible metal centers (see above).

1-His-2-Carboxylate, 2-His-2-Carboxylate, and 3-His-1-Carboxylate Binding Sites. Other types of binding sites, comprising different combinations of His and carboxylate residues, were modeled as well (Figure 2). These mimic the Fe$^{2+}$ active centers in histone deacetylase 8 (His$_{2}$(Asp$_{1}$); binding site; Figure 2a), d-ribulose 5-phosphate 3-epimerase (His$_{5}$(Asp$_{2}$$_{p}$)$_{2}$ binding site; Figure 2b), and S-methylcysteine deaminase (His$_{5}$(Asp$_{2}$); binding site; Figure 2c).

In general, the trends in metal selectivity observed for this group of metal centers follow the tendencies found for the 2-His-1-carboxylate facial triad motif (see above): the binding sites are selective for Fe$^{2+}$ over Mg$^{2+}$ and Mn$^{2+}$ (positive free energies for the Mg$^{2+} \rightarrow$ Fe$^{2+}$ and Mn$^{2+} \rightarrow$ Fe$^{2+}$ exchange; Figure 2) but are ill protected against Zn$^{2+} \rightarrow$ Fe$^{2+}$ substitution (negative $\Delta G$ values for the respective reactions; Figure 2). Solvation has little effect on the process of metal competition.

The relative selectivity of these metal centers, however, varies with their structure and composition. Increasing the number of metal-coordinated imidazole ligands generally enhances the Fe$^{2+}$ over Mg$^{2+}$ selectivity. Thus, $\Delta G^{\text{ex}}$(Mg$^{2+}$ $\rightarrow$ Fe$^{2+}$) increases from 2.9 kcal/mol in the Fe-3wat-1imi-2ace complex (Figure 2a) to 4.7 and 8.1 kcal/mol in Fe-2wat-2imi-2ace (Figure 2b) and Fe-1wat-3imi-1ace (Figure 2c) complexes, respectively. Reducing the Fe$^{2+}$ coordination number to 5 in Fe-1wat-3imi-1ace also contributes to its enhanced selectivity over Mg$^{2+}$. Interestingly, alterations in the complex overall charge, related to the number of metal-bound anionic acetates, do not seem to significantly affect the metal selectivity in these systems. As seen, Fe-3wat-2imi-1ace with an overall charge of 1+ (Figure 1b) exhibits Fe$^{2+}$/Mg$^{2+}$ and Fe$^{2+}$/Mn$^{2+}$ selectivity in the protein environment very similar to that of Fe-2wat-2imi-2ace with an overall charge of 0 (Figure 2b). At the same time, Fe-3wat-2ace and Fe-2wat-2imi-2ace with the same overall charge of 0 are characterized with different metal selectivities (Figure 2a,b). Apparently, the imidazole ligand is more discriminative toward the metal species under study and plays a more substantial role in the selectivity process than its carboxylic counterpart. Furthermore, in analyzing the factors determining the Fe$^{2+}$/Zn$^{2+}$ selectivity, the role of the binding site coordination geometry stands out. Octahedral binding sites (Fe-3wat-1imi-2ace and Fe-2wat-2imi-2ace, Figure 2a,b) are less Zn$^{2+}$/Fe$^{2+}$ selective than pentacoordinated metal centers (Fe-1wat-3imi-1ace, Figure 2c), where lowering the metal coordination number from 6 to 5 favors Zn$^{2+}$ binding to a greater extent than Fe$^{2+}$ binding (more negative $\Delta G$ values in Figure 2c than in Figure 2a,b).

**DISCUSSION**

Central to the proper functioning of metalloproteins is their ability to select with high fidelity the "right" metal cation from the mixture of ions present in the surrounding fluids. Proteins have evolved different selectivity strategies enabling them to preferentially bind the cognate metal cation and protect the active site from attacks by unwanted, non-native contenders, which, if bound, may inactivate the host protein and disrupt the related biochemical reactions. A key selectivity strategy lies in the host protein itself, whose metal binding site features have been finely tuned to account for the subtle differences in the properties of the competing cations. Characteristics of the metal binding site such as its relative rigidity and solvent accessibility, as well as the type, charge, number, orientation, and protonation state of the metal-coordinating ligands, which determine the protein cavity size, geometry, and charge density, influence the metal ion selectivity. In several cases, however, the host protein alone is not able to withstand attacks from other metal cations, which could displace the cognate metal cofactor from the binding site. In such circumstances, it is the cell machinery that orchestrates the selectivity process: metalloregulatory proteins tightly control metal homeostasis.
in living cells by keeping the concentrations of non-native, potentially damaging metal species sufficiently low in comparison to those of the native, less competitive cofactors. Present DFT/PCM calculations shed light on the mechanism of the Fe$^{2+}$–M$^{n+}$ (M = Mg, Mn, Zn) competition in non-heme iron binding sites and disclose the key factors governing the process.

**Fe$^{2+}$–Mg$^{2+}$ Competition.** The results obtained reveal that Mg$^{2+}$ cannot successfully compete with Fe$^{2+}$ for non-heme metal centers, evidenced by the high positive free energies evaluated for the Mg$^{2+}$ → Fe$^{2+}$ exchange (Figures 1 and 2). The major determinant of the high Fe$^{2+}$/Mg$^{2+}$ selectivity in these systems is the composition of the active center, which is invariably lined with His amino acid residues. The “hard” (weakly polarizable) Mg$^{2+}$ cation does not bind as favorably to the “borderline” (more polarizable) histidine residue as its “borderline” Fe$^{2+}$ contender. The histidine residues appear to be more discriminative toward the two competing cations than the respective Asp/Glu ligands and could be considered as the selectivity bearers in these systems (see above). The Fe$^{2+}$ selectivity of the binding site would be further increased if the metal center is rigid and five-coordinated, as opposed to six-coordinated, that substantially disfavors Mg$^{2+}$ from binding, which strongly prefers an octahedral arrangement in its complexes.

**Fe$^{2+}$–Mn$^{2+}$ Competition.** Divalent manganese and iron cations are neighbors in the Irving–Williams series, possessing similar ligand affinities, ion radii, coordination preferences, and cytosolic concentrations, thus appearing to be almost equally strong competitors for protein binding sites. Our calculations reveal, not surprisingly, that iron centers, although still preferably binding Fe$^{2+}$, are weakly selective for Fe$^{2+}$ over Mn$^{2+}$ and are vulnerable to Mn$^{2+}$ attacks. This is evidenced by positive, but low in absolute value, free energies (just a few kcal/mol) of the Mn$^{2+}$ → Fe$^{2+}$ exchange in both the gas phase and protein environment (Figures 1 and 2). The poor Fe$^{2+}$/Mn$^{2+}$ selectivity, resulting in an easily surmountable thermodynamic barrier for the Mn$^{2+}$ → Fe$^{2+}$ substitution, however, might be beneficial for the cell metabolism and/or survival. Under conditions of Fe$^{2+}$ deprivation the iron protein may sequester Mn$^{2+}$ cations from the surrounding milieu which, due to their close resemblance to the native Fe$^{2+}$ ions, secure uninterrupted cell metabolism. Furthermore, Mn$^{2+}$ is not oxidizable and does not react with H$_2$O$_2$, whereas the “catalytic” Fe$^{2+}$ is prone to oxidation by H$_2$O$_2$ (Fenton reaction), causing subsequent inactivation of the respective enzymes. Hence, under conditions of oxidative stress, the Mn$^{2+}$-loaded iron enzymes remain active. In such circumstances, the cell machinery comes into action, ensuring favorable conditions for Mn$^{2+}$ binding by elevating its cytosolic concentration relative to that of Fe$^{2+}$. 

**Fe$^{2+}$–Zn$^{2+}$ Competition.** A zinc cation, characterized by greater ligand affinity than Fe$^{2+}$, can outcompete an iron cation and displace it from its binding sites regardless of their composition and structure (negative free energies for the Zn$^{2+}$ → Fe$^{2+}$ substitution in the entire series of complexes; Figures 1 and 2). The present results are in line with a series of in vitro experiments showing that, indeed, Zn$^{2+}$ binds to the protein with much greater affinity than Fe$^{2+}$. Although the host protein preferentially binds Zn$^{2+}$ in vitro, it is loaded and activated by Fe$^{2+}$ in vivo. Inside the cell, since the protein alone is not able to withstand the attacks by rival Zn$^{2+}$, it is the cell machinery which, by strictly controlling the metal homeostasis and maintaining the free Zn$^{2+}$ concentration at very low levels (in the picomolar to femtomolar range), turns the balance in favor of Fe$^{2+}$. It has been suggested, however, that in critical situations (oxidative stress or Fe$^{2+}$ deficiency) the iron enzyme (which does not operate through a metal redox mechanism) may bind and become activated by Zn$^{2+}$, thus warranting the cell survival.

**CONCLUSIONS**

Various non-heme Fe$^{2+}$ binding sites differing in structure, composition, rigidity, charge state, and solvent exposure have been modeled, and their metal selectivity has been assessed. The results obtained reveal three major factors governing the metal selectivity in these systems, whose interplay determines the outcome of the competition between Fe$^{2+}$ and its contenders:

- the physicochemical properties of the metal cations themselves, reflected in the Irving–Williams series
- the properties of the protein binding site
- the free cytosolic concentration of the competing metal cations.

The present theoretical calculations in combination with available experimental data suggest a dual mechanism for metal selectivity in iron non-heme proteins including internal and external factors. With respect to Mg$^{2+}$, it is the protein itself that controls the selectivity process by suitably optimizing the parameters of its binding site. The structure, rigidity, and composition of the metal center, which are tuned in accordance with the physicochemical properties of the cognate Fe$^{2+}$ cation, appear to be the key descriptors of the Fe$^{2+}$/Mg$^{2+}$ selectivity in these systems. Thus, Fe$^{2+}$ can outcompete Mg$^{2+}$ despite the 3-fold higher free cytosolic concentration of the latter. The iron binding sites are tuned to select Fe$^{2+}$ over Mn$^{2+}$ as well, although the Fe$^{2+}$/Mn$^{2+}$ selectivity is lower than the Fe$^{2+}$/Mg$^{2+}$ selectivity. In the competition between Fe$^{2+}$ and strong transition metals, such as Zn$^{2+}$, however, the control is transferred to the cell machinery, which regulates the selectivity process. The key determinants of metal selectivity in this case are the free metal cytosolic concentrations, which, being tightly controlled by the synchronized action of a number of cell devises, can tilt the balance in favor of the less active “native” cation. Note that the host protein/cell may benefit from the poor or reversed Fe$^{2+}$ selectivity with respect to transition metals (Mn$^{2+}$ or Zn$^{2+}$), as these competitors could substitute for the native cofactor in case of Fe$^{2+}$ deprivation or oxidative stress.

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**Notes**

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