

DFT study of the metal selectivity in protein phosphatases: structural and biomedical implications

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Abstract: Metal ions are essential for the structural stability and catalytic activity of numerous metalloproteins involved in cellular regulation and signaling. Protein phosphatases such as PHLPP2 and PPM1A play a key role in phosphorylation-dependent pathways with direct biomedical relevance, including cancer-related signaling mechanisms. Still, the factors governing metal selectivity in their active sites remain insufficiently understood. In the present study, Density Functional Theory (DFT) calculations are employed to investigate the metal preferences of two structurally distinct phosphatases: PHLPP2, characterized by a mononuclear Zn^{2+} binding site, and PPM1A, containing a binuclear Mn^{2+} catalytic center. The calculations are performed at the B3LYP/6-31+G(3d,p) level of theory to assess the thermodynamics of metal substitution in biologically relevant coordination environments. The results indicate pronounced differences in structural protection and solvent accessibility between the two metal-binding sites, with the Zn^{2+} site in PHLPP2 exhibiting high thermodynamic stability and well-pronounced protection against competing divalent metal ions. In contrast, the binuclear Mn^{2+} center in PPM1A demonstrates greater flexibility and increased susceptibility to metal exchange, particularly in the presence of biologically abundant cations. Overall, the study demonstrates the applicability of DFT calculations as a predictive tool for investigating metal selectivity in metalloproteins and provides further insight into the possible prospects of innovative cancer-treatment strategies in biologically relevant systems.

Keywords: PROTEIN PHOSPHATASES, METAL SELECTIVITY, PHLPP2, PPM1A, METALLOPROTEINS, DFT

1. Introduction

Metal ions play a crucial role in biological systems, contributing to both structural stability and catalytic activity of a wide range of proteins. It is estimated that nearly one third of all proteins require metal ions to function properly, emphasizing the central role of metalloproteins in cellular metabolism, signal transduction, and gene regulation [1,2]. The physicochemical properties of the bound metal ion, along with the surrounding environment formed by the coordinating amino acid residues, determines the stability and reactivity of the active site. Consequently, understanding the main principles that govern the metal selectivity process in proteins remains an important and intriguing objective in bioinorganic chemistry [3].

Metal ion selectivity is determined by both the intrinsic metal properties and the structural features of the protein environment. Factors such as ionic radius, preferred coordination geometry, ligand type, and electrostatic interactions within the binding pocket collectively regulate the stability of metal-protein complexes [2]. In addition to primary coordination sphere, second-shell interactions and solvent accessibility can further significantly affect the metal binding preferences and alter the susceptibility of the metal center to substitution by 'competing' cations present in the cellular environment [4]. Theoretical and computational studies have demonstrated that the protein matrix can stabilize specific metal ions even in the presence of strong thermodynamic competitors [3].

Protein phosphatases constitute an important class of metalloproteins responsible for the removal of phosphate groups from phosphorylated proteins. Hence, they regulate phosphorylation-dependent signaling pathways that control essential cellular processes such as proliferation, differentiation, and apoptosis. Dysregulation of phosphorylation-dephosphorylation cycles has been strongly associated with numerous pathological conditions, including cancer and neurodegenerative diseases [5,6]. Among the metal-dependent phosphatases, members of the PPM family (protein phosphatase Mg^{2+}/Mn^{2+} dependent) play key roles in cellular stress responses and signal transduction pathways [7].

An important representative of the phosphatase family, PPM1A, is a serine/threonine enzyme containing a binuclear catalytic center typically occupied by two Mn^{2+} ions. One of the first deposited structures providing solid evidence on the composition of the active site has been reported in the protein data base under the number 1A6Q [8], which was further used in the current study. Structural and biochemical studies indicate that the two metal ions cooperate during the catalytic process of phosphate hydrolysis by activating a nucleophilic water molecule and stabilizing the transition state [7]. Interestingly, the catalytic metal center of PPM phosphatases has been shown to exhibit a certain degree of flexibility with respect to metal binding, suggesting that substitution by other divalent cations may occur under specific conditions [9].

Another protein of considerable biomedical interest is PHLPP2 (PH domain leucine-rich repeat protein phosphatase 2). Although PHLPP1 and PHLPP2 have previously been reported as protein phosphatases that specifically inactivate Akt by dephosphorylating it [10], we have recently found that purified PHLPP2 has no detectable enzymatic activity in vitro [11], hence it should be considered a pseudophosphatase. Still, it remains a protein of high interest regarding its composition and metal preferences. Structural studies indicate that the catalytic domain of PHLPP2 contains a mononuclear metal-binding site that accommodates a Zn^{2+} ion within its active center modelled in the presented study [11].

Despite extensive experimental investigations, the thermodynamic and structural factors determining metal selectivity in these phosphatases are still not fully understood. In particular, the degree to which the protein environment protects the native metal ion against substitution by other possible divalent cations such as Fe^{2+} or Zn^{2+} present in the cellular milieu remains an open question.

Computational chemistry, and more specifically DFT, offers valuable tools for investigating metal binding and selectivity in biological systems at the molecular level. This quantum chemical approach in complement to experimental data allows for systematic evaluation of the thermodynamic parameters governing metal

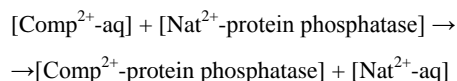
substitution reactions and hence conclude on the stability of a particular active site of interest. Recent studies have demonstrated that DFT-based models can successfully reproduce experimental trends in metal binding affinities and provide valuable insight into the structural determinants of metal selectivity in metalloproteins [3,4].

In the present study, DFT calculations are applied to investigate the metal selectivity of two structurally distinct phosphatases, PHLPP2 and PPM1A. Particular attention is devoted to the thermodynamics of metal substitution reactions involving biologically relevant divalent metal ions. By comparing the behavior of a mononuclear Zn^{2+} binding site with that of a binuclear Mn^{2+} catalytic center, the study aims to elucidate the structural determinants governing metal selectivity and stability in these biologically important enzymes.

2. Computational Methodology

Theoretical calculations were performed using the software package for quantum chemical calculations Gaussian 09 [12]. The geometries of all studied reagents and products were fully optimized by applying the B3LYP functional in combination with the 6-31+G(3d,p) basis set. Frequency calculations for each optimized structure were performed at the same level of theory. No imaginary frequency was found for the lowest energy configurations of any of the optimized entities.

Metal selectivity was evaluated by considering substitution reactions in which the native metal ion bound to the phosphatase is replaced by a competing divalent metal cation. The thermodynamics of these reactions was assessed through the Gibbs energy change associated with the following general process:



where Nat^{2+} denotes the native metal ion and Comp^{2+} represents the competing metal species. Open-shell Fe^{2+} and Mn^{2+} cations were modeled as high-spin species, quintuplet and sextuplet, respectively, in line with the experimental and theoretical findings [13,14].

The differences ΔE_{el} , ΔE_{th} , and ΔS between the products and reactants were used to calculate the Gibbs energy of metal competition ΔG in the gas phase (denoted by superscript 1) at room temperature (298.15 K and 1 atm pressure), according to the equations:

$$\Delta H = \Delta E_{el} + \Delta E_{th} + P\Delta V \quad (1)$$

$$\Delta G^1 = \Delta H - T\Delta S \quad (2)$$

In equation (1), ΔH is enthalpy, ΔE_{el} is electronic energy and ΔE_{th} is thermal energy. All energies are in kcal mol^{-1} . $P\Delta V$ is a work term that takes into account the difference in the number of the moles of the reactant(s) and moles of the product(s) and its value equals “-0.59” for $\Delta n=1$. The Gibbs energy in the gas phase, ΔG^1 , is calculated using equation (2). The influence of the solvent on the thermodynamics of the investigated processes was evaluated using the SMD [15] method in solvents diethyl ether ($\epsilon \approx 4$) and propanonitrile ($\epsilon \approx 29$). These are the media of choice when simulating a closed-shell active site (diethyl ether), or a relatively open to the solvent active site (propanonitrile). The energy of metal substitution in a medium characterized by dielectric constants of $\epsilon=4$ and $\epsilon=29$, ΔG^4 and ΔG^{29} respectively, is obtained by equation (3):

$$\Delta G^\epsilon = \Delta G^1 + \Delta G_{solv}^\epsilon (\text{Products}) - \Delta G_{solv}^\epsilon (\text{Reactants}) \quad (3)$$

The change in Gibbs energy provides useful information about the reaction's energetics and spontaneity: reactions with a

negative value of ΔG can happen without an energy input, and are denoted as spontaneous or exergonic, while those with a positive ΔG are referred to as endergonic and are not expected to occur spontaneously from thermodynamic point of view. Therefore, in the frame of the current study a modeled reaction with $\Delta G < 0$ corresponds to a plausible substitution (a metal binding site prone to metal exchange), whereas in the case of a positive outcome ($\Delta G > 0$) the metal center is protected against an outer attack.

3. Results and Discussion

The calculated thermodynamic parameters reveal pronounced differences in the stability and metal selectivity of the catalytic centers of the two investigated phosphatases. For the mononuclear catalytic site of PHLPP2, the calculations indicate a clear thermodynamic preference for the native zinc ion over the competing divalent metal ions taken into consideration - Mn^{2+} or Fe^{2+} . Replacement of Zn^{2+} by either a ferrous, or a manganese ion is associated with positive Gibbs energy changes in both environments under study, indicating that substitution of the native metal ion is thermodynamically unfavorable. The obtained results for the active site of PHLPP2 are summarized in Figure 1.

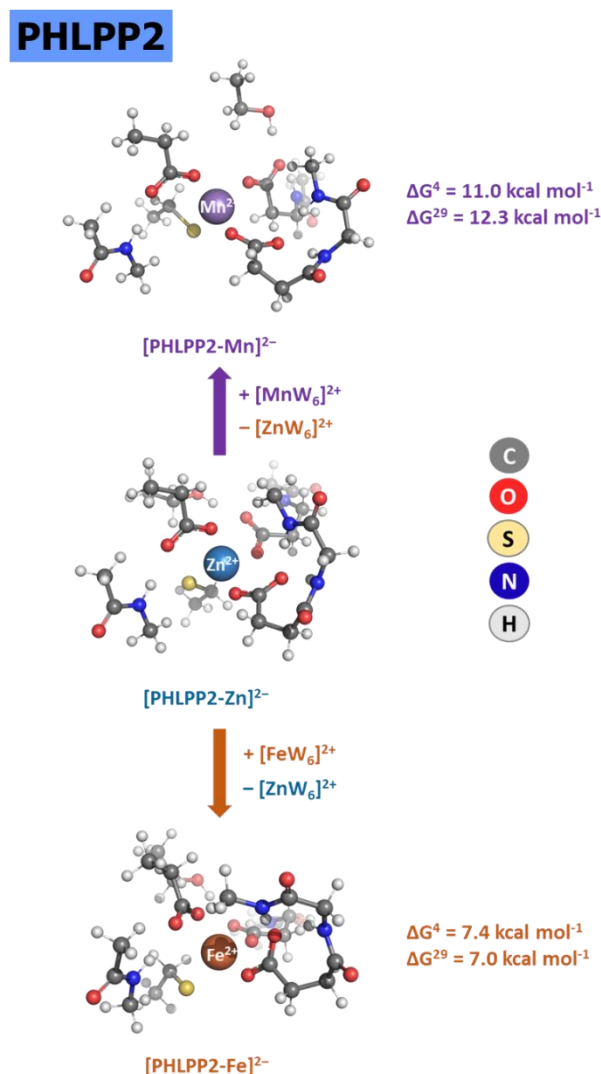


Fig. 1 B3LYP/6-31+G(3d,p) optimized geometries of a simplified model of the PHLPP2 catalytic site illustrating the thermodynamics of Zn^{2+} substitution by Mn^{2+} and Fe^{2+} , with the corresponding ΔG at dielectric constants $\epsilon = 4$ and $\epsilon = 29$.

The calculated Gibbs energies for replacing a zinc ion with either a manganese or a ferrous one stand firmly on positive ground in both modelled environments: $\Delta G^{4/29} = 11.0/ 12.3 \text{ kcal}$

mol^{-1} for a $\text{Zn}^{2+} / \text{Mn}^{2+}$ replacement, and $\Delta G^{4/29} = 7.4 / 7.0 \text{ kcal mol}^{-1}$ for a $\text{Zn}^{2+} / \text{Fe}^{2+}$ substitution, respectively. These results indicate that the Zn^{2+} binding site in PHLPP2 is well-protected against an outer attack due to the amino-acid composition of the active site provided by the surrounding protein matrix. Moreover, the calculated Gibbs energies in the two surrounding media at $\epsilon = 4$ and $\epsilon = 29$ differ by just about 0.4 to 1.3 kcal mol^{-1} , thus indicating that the metal selectivity of the PHLPP2 catalytic site is not affected by exposition to the solvent. This outcome corresponds to a structurally protected metal-binding pocket within the protein interior.

On the other hand, the binuclear catalytic center of PPM1A displays a markedly different thermodynamic behavior. The calculated Gibbs energies of metal replacement in the modeled PPM1A active site are presented in Figure 2.

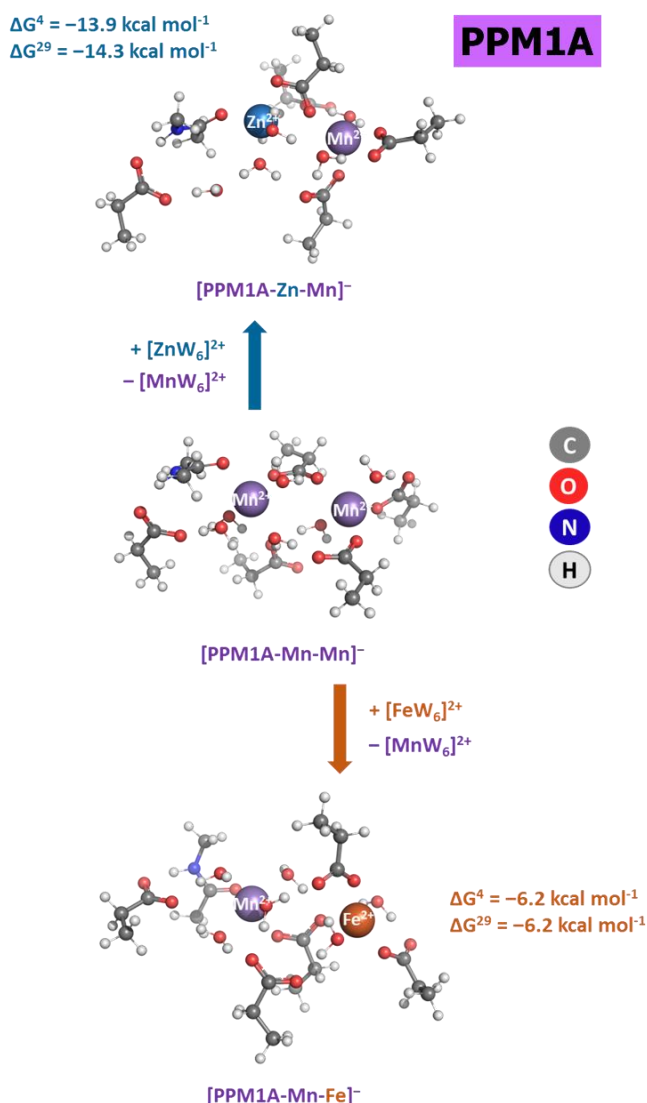


Fig. 2 B3LYP/6-31+G(3d,p) optimized geometries of a simplified model of the PPM1A catalytic site illustrating the thermodynamics of Mn^{2+} substitution by Zn^{2+} and Fe^{2+} , with the corresponding ΔG at dielectric constants $\epsilon = 4$ and $\epsilon = 29$.

The calculated Gibbs energies indicate that replacement of the native Mn^{2+} ions by Zn^{2+} or Fe^{2+} is thermodynamically favorable in both dielectric environments. In particular, substitution by Zn^{2+} is associated with strongly negative Gibbs energy values of $\Delta G = -13.9 \text{ kcal mol}^{-1}$ in a protein enclosed environment ($\epsilon = 4$)

and $\Delta G = -14.3 \text{ kcal mol}^{-1}$ in a partially solvent accessible medium ($\epsilon = 29$). Furthermore, replacement of Mn^{2+} by Fe^{2+} is also thermodynamically favorable, with calculated values of $\Delta G^{4/29} = -6.2 \text{ kcal mol}^{-1}$ at both dielectric constants. These results indicate that unlike the Zn^{2+} site of PHLPP2, the binuclear metal center of PPM1A is prone to metal substitution. The obtained negative $\Delta G^{4/29}$ values provide strong theoretical evidence of the susceptibility of the catalytic center of PPM1A to metal exchange, which should be attributed to the amino acid composition of the active site.

The obtained theoretical data strongly correspond to experimental observations showing that Zn^{2+} can indeed inhibit PPM1A activity by displacing the native Mn^{2+} ions under in vitro conditions. Nevertheless, under physiological conditions the intracellular concentration of free Zn^{2+} ions in the cytosol is maintained at extremely low levels ($10^{-12} - 10^{-15} \text{ M}$, [2]), whereas Mn^{2+} ions are present at higher concentrations (10^{-6} M , [2]). Consequently, Mn^{2+} remains the preferred catalytic metal in vivo despite the thermodynamic feasibility of substitution observed in the calculations.

The calculations additionally suggest that Fe^{2+} could potentially substitute for Mn^{2+} within the binuclear catalytic center of PPM1A. Because intracellular concentrations of Fe^{2+} and Mn^{2+} may be comparable under certain cellular conditions (in micromolar range [2]), Fe^{2+} may serve as an alternative catalytic metal when Mn^{2+} availability becomes limited.

Taken together, the results highlight the critical role of structural protection and solvent accessibility in determining metal selectivity in metalloproteins. While the Zn^{2+} binding site of PHLPP2 appears to be strongly stabilized by the surrounding protein environment, the catalytic center of PPM1A exhibits a higher susceptibility to metal exchange processes.

4. Conclusions

The present study employs DFT calculations to investigate the metal selectivity of two structurally distinct protein phosphatases, PHLPP2 and PPM1A. The results reveal marked differences in the thermodynamic stability and susceptibility to metal substitution between the two catalytic centers. The mononuclear Zn^{2+} binding site in PHLPP2 exhibits a strong thermodynamic preference for the native metal ion and appears to be well protected against substitution by competing divalent cations. In contrast, the binuclear Mn^{2+} catalytic center of PPM1A shows greater susceptibility to metal exchange, particularly in environments characterized by higher dielectric constants.

These findings highlight the importance of protein architecture and solvent accessibility in controlling metal selectivity in metalloproteins. Overall, the results demonstrate that DFT calculations can provide valuable molecular-level insight into the factors governing metal binding and substitution processes in biologically relevant systems.

5. References

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