Journal of Molecular Graphics and Modelling 73 (2017) 24–29

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

journal homepage: www.elsevier.com/locate/JMGM

Topical Perspectives

Effects of metal-ion replacement on pyrazinamidase activity: A quantum mechanical study

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ARTICLE INFO

Article history:
Received 8 November 2016
Received in revised form 27 January 2017
Accepted 30 January 2017
Available online 2 February 2017

Keywords:
Pyrazinamidase
Tuberculosis
Quantum mechanics
Metalloenzymes

ABSTRACT

Pyrazinamidase (PZase), a metalloenzyme, is responsible for acidic modification of pyrazinamide (PZA), a drug used in tuberculosis treatment. The metal coordination site of the enzyme is able to coordinate various divalent metal cofactors. Previous experimental studies have demonstrated that metal ions, such as Co^{2+}, Mn^{2+}, and Zn^{2+}, are able to reactivate metal-depleted PZase, while others including Cu^{2+}, Fe^{2+}, and Mg^{2+}, cannot restore activity. In this study, we investigated binding of various metal ions to the metal coordination site (MCS) of the enzyme using quantum mechanical calculations. We calculated the metal–ligand (residue) binding energy and the atomic partial charges in the presence of various ions. The results indicated that the tendency of alkaline earth metals to bind to PZase MCS is very low and not suitable for enzyme structural and catalytic function. In contrast, Co^{2+} and Ni^{2+} ions have very high binding affinity and are favorable to the structural and functional properties of the enzyme. Furthermore, we observed that the rate at which Ni^{2+}, Co^{2+} and Fe^{2+} ions in PZase MCS polarize the O—H bond of coordinated water molecules is much higher than the polarization rate created by other ions. This finding suggests that the coordination of Ni^{2+}, Co^{2+}, or Fe^{2+} to PZase facilitates the deprotonation of coordinated water molecules to generate a nucleophile that catalyzes the enzymatic reaction.

1. Introduction

Metalloenzymes are enzymes that contain metal ions that catalyze a variety of reactions with high selectivity and activity under mild conditions [1–7]. Therefore, the presence of metal ions as cofactors in the active site of enzymes plays an important role in their biological activity. Metal ions can add new functionalities to an enzyme and perform vital reactions in biological processes. It has been estimated that more than one third of all natural enzymes are metalloenzymes and contain one of the following ten metal ions: Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} [4,8–11]. It has also been stated in previous studies that metal ions such as iron, zinc, copper, calcium, nickel, and magnesium, play various roles in protein structure and function [5,12–15]. These ions are responsible for many vital functions, *e.g.*, enhanced structural stability, oxygen storage, catalytic processes of enzymes, and electron transfer [3,16,17]. However, the effective roles of metal ions are usually elicited not by the isolated forms of the ions themselves, but by their capitalization while bonded to coordinating ligands. The coordination of metal ions in metalloenzymes affects the local electron distribution and effectively controls the catalytic reaction [18–20].

For this study, we chose to investigate the *Mycobacterium tuberculosis* pyrazinamidase (PZase). PZase metabolizes the antibiotic pyrazinamide (PZA) a critical first-line drug in Tuberculosis (TB) treatment, into its active form, pyrazinoic acid (POA) [21–23]. Previous studies have shown that PZase is a metalloenzyme and that the metal coordination site (MCS) is necessary for its activity [24–27]. Based on the three-dimensional structure of *M. tuberculosis* PZase, Petrella et al. revealed that the iron ion of PZase is coordinated by a conserved binding tetrad of amino acids consisting of one aspartate, three histidine residues, as well as two-coordinated water molecules [25]. Sheen et al. have shown that metal-depleted *M. tuberculosis* PZase shows different reactivation patterns depending on the metal ion substituted [28]. These researchers also found that among a range of metal ions, Co^{2+}, Mn^{2+} and Zn^{2+}, could reactivate the metal-depleted PZase [28]. It has also been reported that the metal-depleted PZase was reactivated by Mn^{2+} and Fe^{2+} [29]. How-

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http://dx.doi.org/10.1016/j.jmgm.2017.01.021
1093-3263/

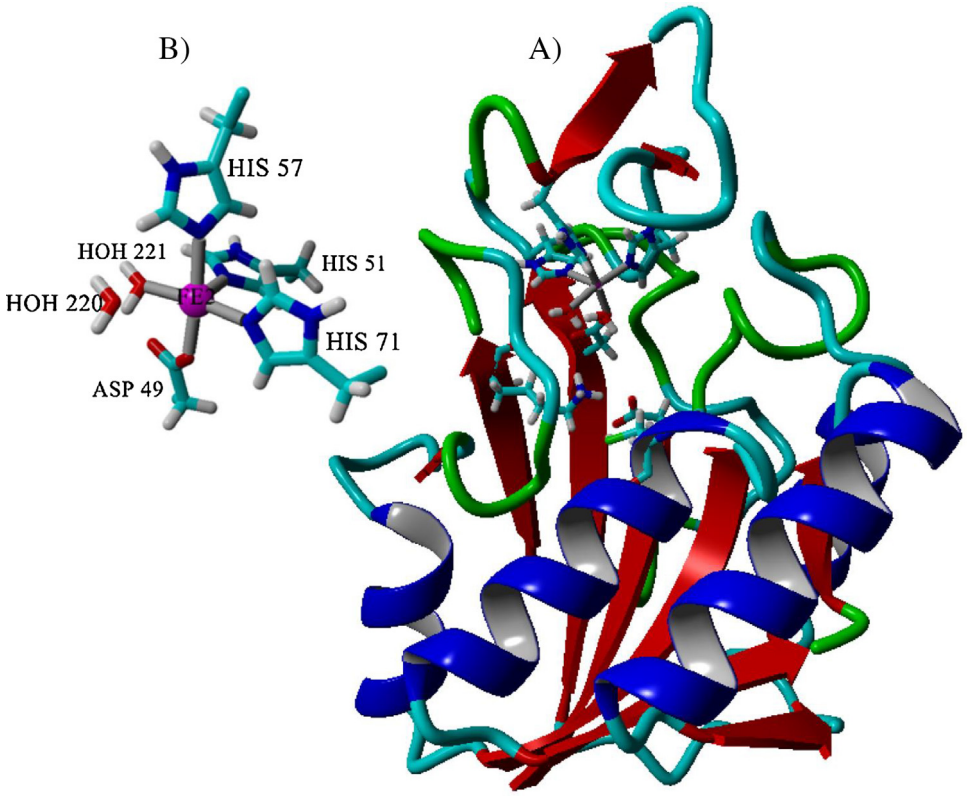

Fig. 1. A) Pyrazinamidase and B) metal coordination site (MCS).

ever, the exact role of the metal ions in PZase is not fully understood. Previous observations have shown that mutations in the active site or the metal-binding site affected PZase activity, whereas mutations far from these sites decreased enzyme activity to varying levels [24,29–33]. PZase can accept various metal ions as a cofactor, and each ion affects activity differently.

As outlined above, there is no set of rules that explains how metal ions will behave in an enzyme. Therefore, biophysics and related disciplines work to understand metalloprotein activity, specifically the role of the metal ions. Improvements in molecular modeling have become valuable tools to access the structural and mechanistic details of metal-binding proteins that experimental methods cannot explore. Indeed, molecular modeling may access resolutions (atomic) and time scales (fs) that are often not accessible experimentally. In this study, we chose a density functional theory (DFT) method that enables the exploration of biological systems at an atomic level and can potentially provide valuable insight into these systems' properties [34–38]. In this study, the presence of eight divalent metal ions in PZase- Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} –and how they affect the enzyme's properties has been computationally investigated. First, we calculated the dipole moment of metal-coordinated water molecules, the partial charge in the aqueous phase, the metal-ligand bond length, the ordering of metal-ligand binding energy and the free energy of the iron-containing model. Next, we substituted Fe^{2+} with Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} in MCS and repeated the calculations. These quantum mechanical computations allow us to compare the affinity of ions to the MCS, which could be helpful in substrate binding and enzyme reactivity studies.

2. Computational methods

The *M. tuberculosis* PZase MCS consists of four amino-acid residues (Asp49, His51, His57, and His71), two water molecules (H_2O220 and H_2O221), and a divalent metal atom (Fig. 1). The initial MCS structure was obtained from the crystallized PZase structure of the *M. tuberculosis* H37Rv strain (PDB ID: 3PL1) [25], and the end of cleaved bonds were capped with hydrogen atoms. In the crystalline structure, the MCS is coordinated with Fe^{2+}. Using this MCS-Fe^{2+} configuration, we constructed seven additional complexes by substitution of Fe^{2+} ion with Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}. All quantum mechanical calculations were performed using the ORCA version 3.0.1 package [39]. For geometry optimization and single point energy calculations, the Density Functional Theory (DFT) [40,41] with different exchange and correlation potentials have been used. In particular for the exchange contributions, we have employed the functionals proposed by Perdew and Wang (PW) [42] and Becke (B and B3) [43,44], while for the correlation the Perdew (P86 and P91) [45,46] and the Lee, Yang and Parr (LYP) [47] functionals have been employed. All combinations of exchange and correlation functionals are possible for a typical DFT based calculation. Meanwhile, PWP [45,48], PB86 [43,45] and B3LYP [44,47] are the most established methods and we have employed these methods to show the validity and reliability of our calculated data. We also used the Pople-style polarization function 6-31G(d,p) [49] basis set for the C, H, O, and N; and the Los Alamos National Laboratory (LANL2DZ) [50] basis set with effective core potentials (ECP) for the metal atoms [51,52]. In the ECP, the core electrons were replaced by an effective potential that speeds calculations with little loss of accuracy. The eight complexes were optimized in gas- and aqueous-phase systems using the Conductor-like Screening Model (COSMO) [53]. COSMO imitates the water solvent effect on the compounds as a continuum with a permittivity, and therefore is a continuum solvation model. Unlike other continuum solvation models, the COSMO model obtains the polarization charges of the continuum (caused by the polarity of the solute) from a scaled-conductor approximation. The ChelpG method was used to determine equivalent charges