In silico design of potentially functional artificial metallo-haloalkane dehalogenase containing catalytic zinc

ABSTRACT

Artificial metalloenzymes are unique as they combine the good features of homogeneous and enzymatic catalysts, and they can potentially improve some difficult catalytic assays. This study reports a method that can be used to create an artificial metal-binding site prior to proving it to be functional in a wet lab. Haloalkane dehalogenase was grafted into a metal-binding site to form an artificial metallo-haloalkane dehalogenase and was studied for its potential functionalities in silico. Computational protocols regarding dynamic metal docking were studied using native metalloenzymes and functional artificial metalloenzymes. Using YASARA Structure, a simulation box covering template structure was created to be filled with water molecules followed by one mutated water molecule closest to the metal-binding site to metal ion. A simple energy minimization step was subsequently run using an AMBER force field to allow the metal ion to interact with the metal-binding residues. Long molecular dynamic simulation using YASARA Structure was performed to analyze the stability of the metal-binding site and the distance between metal-binding residues. Metal ions fluctuating around 2.0 Å across a 20 ns simulation indicated a stable metal-binding site. Metal-binding energies were predicted using FoldX, with a native metalloenzyme (carbonic anhydrase) scoring 18.0 kcal/mol and the best mutant model (C1a) scoring 16.4 kcal/mol. Analysis of the metal-binding site geometry was performed using CheckMyMetal, and all scores for the metalloenzymes and mutant models were in an acceptable range. Like native metalloenzymes, the metal-binding site of C1a was supported by residues in the second coordination shell to maintain a more coordinated metal-binding site. Short-chain multihalogenated alkanes (1,2-dibromoethane and 1,2,3-trichloropropane) were able to dock in the active site of C1a. The halides of the substrate were in contact with both the metal and halide-stabilizing residues, thus indicating a better stabilization of the substrate. The simple catalytic mechanism proposed is that the metal ion interacted with halogen and polarized the carbon–halogen bond, thus making the alpha carbon susceptible to attack by nucleophilic hydroxide. The interaction between halogen in the metal ion and halide-stabilizing residues may help to improve the stabilization of the substrate–enzyme complex and reduce the activation energy. This study reports a modified dynamic metal-docking protocol and validation tests to verify the metal-binding site. These approaches can be applied to design different kinds of artificial metalloenzymes or metal-binding sites.

Keyword: Artificial metallo-haloalkane dehalogenase; Artificial metalloenzyme; Dynamic metal docking