Computational Analysis of the Evolution of Glutathione Peroxidase 6 (GPX6) Activation Free Energy

Nayanika Das,[†] Gunjan Paul,[‡] Vijay Baladhye,[¶] and Jordi Villà-Freixa[*,†,§]

†*Computational Biochemistry and Biophysics Lab, Research Group on Bioinformatics and Bioimaging (BI^2), Department of Biosciences, Universitat de Vic - Universitat Central de Catalunya, 08500 Vic, Spain*

‡*University Autonomous de Barcelona*

¶*Savitribai Phule Pune University, Pune, India*

§*IRIS-CC*

E-mail: jordi.villa@uvic.cat

Abstract

Outstanding success in computational protein design has been achieved in recent years by combining machine learning approaches with physicochemical property analysis of protein variants. Despite these efforts, optimization of enzyme activity remains challenging. Here, we propose a method combining the Empirical Valence Bond (EVB) method with computational free energy calculations to evaluate mutational pathways leading to optimized enzyme activity. Using GPX6 as a model, we compute activation free energy differences between the human (selenocysteine-containing) and mouse (cysteine-containing) orthologs. Our results provide insights into the evolutionary dynamics of selenium usage in enzymes.

Introduction

Selenium (Se), in the form of selenocysteine (Sec, U), the 21st amino acid, is found in 25 human proteins. Sec insertion into proteins involves recoding the UGA stop codon as a sense codon, a process requiring unique biological machinery
citeHondal2011. The chemical advantages of Sec over cysteine (Cys) include its enhanced nucleophilic character and a much lower pKa, making it more suitable for redox reactions citeHondal2011,Cardey2007. These properties are critical in enzymes such as glutathione peroxidases (GPXs), which protect cells from oxidative stress by catalyzing the reduction of peroxides.

Mechanism of GPX

The catalytic mechanism of GPX has been extensively studied. A mechanism proposed for GPX3 by Prabhakar et al., using DFT calculations, involves the selenol form of Sec reacting with hydrogen peroxide, with a computed activation barrier of 16.4 kcal/mol
citePrabhakar2006. Other studies suggest alternative pathways, such as proton transfer via water or direct reduction by the selenolate form
citeOrian2015, Flohe2022. These studies highlight the flexibility and efficiency of Sec in catalysis.

Empirical Valence Bond Model

The EVB model offers a computationally efficient alternative to quantum mechanics/molecular mechanics (QM/MM) approaches for simulating enzymatic reactions. The EVB potential energy is defined as:

$$E_{EVB} = E_0 + \sum_{i=1}^{N} \frac{1}{2} k_i \Delta x_i^2 + V_0, \tag{1}$$

where Δx_i represents deviations of reaction coordinates, E_0 is the system's equilibrium energy, and V_0 is a constant. This approach captures environmental effects on reaction energetics, enabling efficient analysis of enzyme evolution and mutational pathways citeCarvalho2014.

Results and Discussion

Using EVB, we calculated the activation free energy for the GPX6-catalyzed reaction in both human (Sec) and mouse (Cys) variants. Our results show significant differences in activation barriers, highlighting the role of Sec in optimizing enzyme function. Additionally, mutational pathways derived from our simulations suggest evolutionary strategies that preserve catalytic efficiency while adapting to environmental pressures.

Conclusions

This study demonstrates the utility of EVB modeling in exploring enzyme evolution and optimizing catalytic activity. By analyzing the activation free energy of GPX6 variants, we provide insights into the functional advantages of selenium-based catalysis and propose computational strategies for enzyme engineering.