Phosphatase Activity of Haloacid Dehalogenase Subfamily from *Klebsiella pneumoniae* expressed in *Escherichia coli* BL21 (DE3)/pET-*hakp2* Recombinant Clone

PPMI Hibah Penelitian dan Inovasi KK

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Abstract

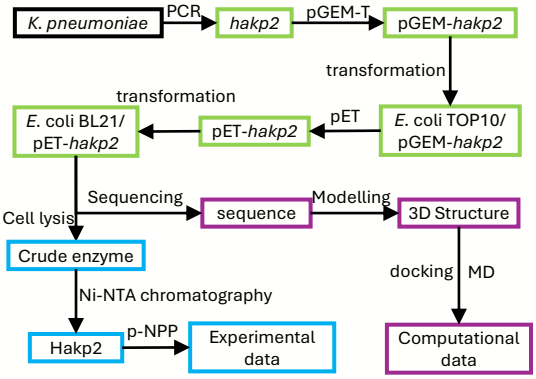
Haloacid dehalogenase (HAD) is a superfamily of enzymes grouped by their structural similarity. Despite the name, some HAD enzymes catalyze the cleavage of phosphoester bonds and are classified as HAD phosphatases. Hakp, a HAD enzyme isolated from *Klebsiella pneumoniae* ITB1, shares a conserved catalytic motif with known HAD phosphatases, suggesting that it may also possess phosphatase activity. This study aimed to isolate, clone, express, and characterize a HAD phosphatase (Hakp2) from K. pneumoniae ITB2, and to evaluate its phosphatase activity both computationally and experimentally. The hakp2 gene was isolated from the chromosomal DNA of K. pneumoniae ITB2, cloned into the pGEM-T vector, subcloned into the pET-30a(+) expression vector, and expressed in E. coli BL21(DE3). Molecular dynamics simulation indicated that the Mg2+ ion plays a key role in stabilizing the substrate within the catalytic site. Experimentally, Hakp2 was active only in the presence of Mg2+ and exhibited optimal activity at pH 5 (39.9 U/mg, 37 °C) and at 45 °C (73.1 U/mg, pH 7).

Keyword: Phosphatase, Haloacid Dehalogenase, *Klebsiella pneumoniae*

Introduction

Haloacid dehalogenase (HAD) is a large superfamily of enzyme comprised mostly of hydrolases. Two important subclasses of HAD are HAD phosphatases, and HAD dehalogenases. These enzymes are grouped by the similarity of their three-dimensional structure. Klebsiella pneumoniae was reported to possess a haloacid dehalogenase in which, showed sequence similarity to HAD phosphatases. Most HAD phosphatase emerged to have DXDXT(L/V) motif at their active site, whereas dehalogenase from K. pneumoniae ITB1 has a DXEXTT sequence that more closely resembles the HAD phosphatase motif. E and D residues have a similar properties being a negatively charged residue, suggesting that the HAD from this bacterium may also act as phosphatase, providing a basis for further investigation that could expand the potential applications of this enzyme.

Research Method



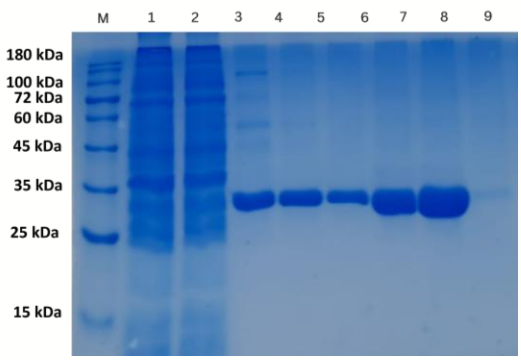


***Figure 1**. SDS PAGE of Hakp2 purification fractions*

Discussion & Result

Purified Hakp2 was analyzed by polyacrylamide gel electrophoresis, which showed a single band at approximately 30 kDa (**Figure 1**). The concentration of purified Hakp2 was determined using nano spectrophotometer at 280 nm and the concentration of Hakp2 is 0.52 mg/mL. This method resulted in a moderate protein recovery of 60% and 11.15-fold purification was achieved.

The structure of Hakp2 (**Figure 2**) was docked with p-NPP in the presence of Mg^{2+} ion. The presence of Mg^{2+} ion stabilized the position of p-NPP with the formation of salt-bridge interaction (**Figure 3**). The addition of Mg2+ ion greatly lowered the ΔGbind, further stabilizing the position of p-NPP in the catalytic site.

The phosphatase activity of Hakp2 towards p-NPP with the presence of Mg^{2+} is shown in **Figure 3**. Hakp2 shows the highest activity in pH 5 while retaining substantial activity under basic condition. This indicates that Hakp2 has a relatively broad pH tolerance from acidic pH of 5 to basic pH. Hakp2 shows the highest activity at 45 ℃ with a sharp decline at 50 ℃ and 55 ℃ indicating a low thermal stability.

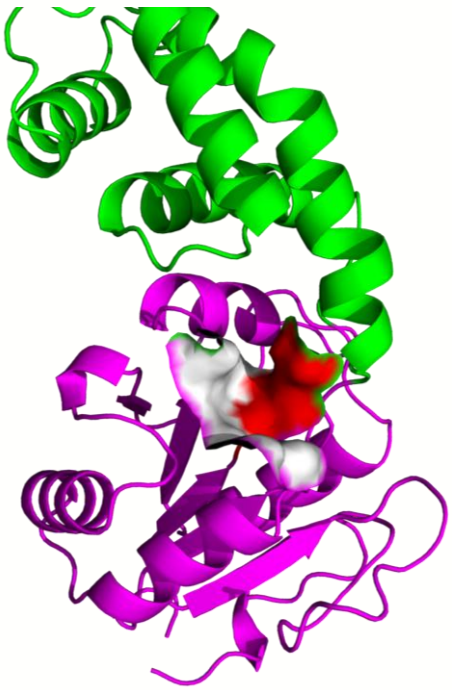


***Figure 2**. 3D structure of Hakp2*

Conclusion

Hakp2 has a dephosphorylating activity. Computational analysis shows that Hakp2 can accept p-NPP as a substrate with the help of Mg2+ ion forming a salt bridge with the negatively charged p-NPP. Experimentally, Hakp2 showed broad pH tolerance at 37 ℃ ranging from acidic (5 and 6) to basic (7 and 8) solution, while retaining around half of the activity in pH 9. On the other hand, Hakp2 shows the highest activity in 45 ℃ and a significant drop of activity in 50 ℃ and 55 ℃.

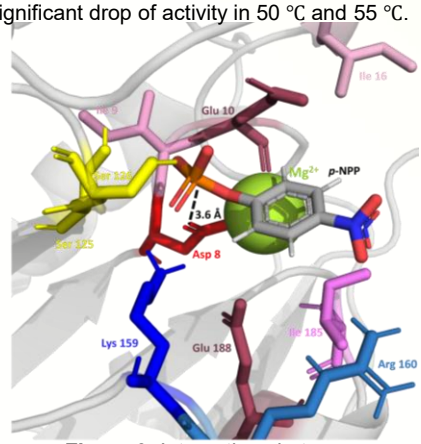


***Figure 3**. Interactions between Hakp2 and p-NPP*

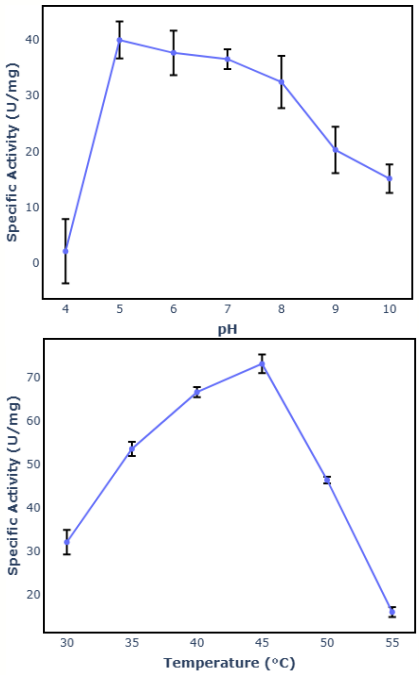


***Figure 4**. Specific activity of Hakp2 under different conditions*