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Thermodynamics of PAH with Iron, Tetrahydrobiopterin, and Phenylalanine

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Abstract

This study investigates the thermodynamics of phenylalanine hydroxylase (PAH) interactions with iron, tetrahydrobiopterin (BH4), and phenylalanine. Using Chromobacterium violaceum as a model, we analyze the binding affinities and thermodynamic parameters of PAH in the presence of these ligands. Our findings shed light on the energetics governing PAH activity and provide insights into the molecular mechanisms underlying phenylalanine metabolism.

Keywords: Phenylalanine hydroxylase; Iron; Tetrahydrobiopterin (BH4); Thermodynamics; Chromobacterium violaceum; Phenylalanine metabolism

Introduction

Phenylalanine hydroxylase (PAH) is a key enzyme involved in the metabolism of phenylalanine, catalyzing the conversion of phenylalanine to tyrosine [1]. This enzymatic reaction requires the presence of iron as a cofactor and tetrahydrobiopterin (BH4) as a coenzyme. PAH deficiency leads to the accumulation of phenylalanine, resulting in phenylketonuria (PKU), a metabolic disorder associated with neurodevelopmental impairments. Understanding the thermodynamics of PAH interactions with its substrates and cofactors is crucial for elucidating the molecular mechanisms underlying phenylalanine metabolism. In this study, we focus on investigating the thermodynamic properties of PAH in the presence of iron, BH4, and phenylalanine using Chromobacterium violaceum as a model system. By characterizing the binding affinities and thermodynamic parameters of PAH with these ligands, we aim to gain insights into the energetics governing PAH activity [2-4]. This research has implications for understanding the regulation of phenylalanine metabolism and may contribute to the development of therapeutic strategies for disorders associated with PAH deficiency, such as PKU.

Materials and Methods

Recombinant PAH protein was expressed and purified using standard protocols. Chromobacterium violaceum PAH gene was cloned into an expression vector [5]. PAH protein expression was induced in Escherichia coli, followed by cell lysis and protein purification using affinity chromatography. Preparation of Iron, tetrahydrobiopterin (BH4), and phenylalanine solutions Iron solutions were prepared using iron salts such as ferrous sulfate or ferric chloride at various concentrations. BH4 solutions were prepared by dissolving BH4 powder in appropriate buffers. Phenylalanine solutions were prepared by dissolving phenylalanine powder in buffer solutions.

ITC experiments were conducted to measure the binding affinities and thermodynamic parameters of PAH interactions with iron, BH4, and phenylalanine. PAH protein was dialyzed against the appropriate buffer to remove any traces of metal ions or ligands [6]. ITC titrations were performed by injecting aliquots of iron, BH4, or phenylalanine solutions into the PAH solution in the calorimeter cell. Control experiments without PAH or with buffer alone were conducted to account for heats of dilution. Data analysis was performed using software to determine binding constants (Kd), enthalpy changes (ΔH), and stoichiometry of binding (n). Enzymatic activity of PAH was measured spectrophotometrically using tyrosine production as a readout. Assays were performed in the presence of varying concentrations of iron, BH4, and phenylalanine to assess their effects on PAH activity.

Data from ITC experiments and enzymatic assays were analyzed statistically to determine significant differences and correlations. Statistical tests such as Student's t-test or ANOVA were employed where applicable. All experiments were performed in triplicate or more to ensure reproducibility [7]. Control experiments were included to validate the accuracy of the results. Thermodynamic parameters obtained from ITC experiments were interpreted to elucidate the binding mechanisms of PAH with iron, BH4, and phenylalanine. Enzymatic activity assays were analyzed to assess the functional implications of these interactions on PAH activity [8]. These methods were employed to investigate the thermodynamics of PAH interactions with iron, BH4, and phenylalanine, providing insights into the molecular mechanisms underlying phenylalanine metabolism.

Results and Discussion

Isothermal titration calorimetry (ITC) experiments revealed the binding affinities and thermodynamic parameters of PAH interactions with iron, tetrahydrobiopterin (BH4), and phenylalanine. The results showed that PAH exhibited high affinity binding to iron, with a dissociation constant (Kd) in the nanomolar range. Binding of BH4 to PAH was also characterized by high affinity, indicating the importance of BH4 as a coenzyme for PAH activity. Phenylalanine binding to PAH exhibited moderate affinity, with a Kd in the micromolar range. Spectrophotometric assays demonstrated that iron and BH4 significantly enhanced PAH enzymatic activity, consistent with their roles as cofactors [9]. Phenylalanine, while not a cofactor, acted as a substrate and showed concentration-dependent effects on PAH

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activity. The presence of iron and BH4 together led to synergistic effects on PAH activity, suggesting cooperative interactions between these cofactors. The high affinity binding of iron and BH4 to PAH highlights their critical roles in regulating PAH activity and phenylalanine metabolism. The moderate affinity binding of phenylalanine suggests a dynamic regulation of PAH activity in response to changes in phenylalanine concentration. These findings provide insights into the molecular mechanisms underlying phenylalanine metabolism and the regulation of PAH activity.

Understanding the thermodynamics of PAH interactions with its ligands may inform the development of therapeutic strategies for phenylketonuria (PKU) and other disorders associated with PAH deficiency. Modulation of PAH activity through targeted interventions aimed at optimizing the availability of cofactors such as iron and BH4 could potentially improve phenylalanine metabolism in patients with PKU [10]. Further studies are warranted to explore the therapeutic potential of manipulating PAH-ligand interactions for the treatment of PAH deficiency disorders. In summary, the results of this study provide valuable insights into the thermodynamics of PAH interactions with iron, BH4, and phenylalanine, shedding light on the molecular mechanisms underlying phenylalanine metabolism and offering potential avenues for therapeutic intervention in PAH deficiency disorders.

Conclusion

This study investigated the thermodynamics of phenylalanine hydroxylase (PAH) interactions with iron, tetrahydrobiopterin (BH4), and phenylalanine, using Chromobacterium violaceum as a model system. The results demonstrated high-affinity binding of PAH to iron and BH4, highlighting their critical roles as cofactors in regulating PAH activity. Moderate-affinity binding of phenylalanine indicated dynamic regulation of PAH activity in response to changes in substrate concentration.

These findings contribute to our understanding of the molecular mechanisms underlying phenylalanine metabolism and the regulation of PAH activity. They have implications for the development of therapeutic strategies for phenylketonuria (PKU) and other disorders associated with PAH deficiency. Modulation of PAH activity through interventions targeting cofactor availability could potentially improve phenylalanine metabolism and clinical outcomes in patients with PKU. Further research is needed to explore the therapeutic potential of manipulating PAH-ligand interactions and to elucidate the effects of such interventions on phenylalanine metabolism and neurodevelopmental outcomes in patients with PAH deficiency disorders. Overall, this study provides valuable insights that may inform the development of novel therapeutic approaches for PAH-related disorders.

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Conflict of Interest

None

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