Characterization of Recombinant Human Alpha/Beta-Hydrolase Domain 6 Using Ligand Assisted Protein Structure Approach

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Abstract

The serine α/β-hydrolase domain 6 (ABHD6) enzyme, a new member of the endocannabinoid system, hydrolyzes the lipid signaling molecule 2-arachidonyl glycerol both in the CNS and periphery. Mounting evidence connects selective ABHD6 inhibition and positive outcomes for several diseases like metabolic disorders, inflammation, and neurodegenerative diseases. ABHD6 becomes an attractive target for therapeutic intervention, yet, its role is not well-characterized and its three-dimensional structure is lacking. Several ABHD6 constructs were developed for expression and purification of functional hABHD6 variants for biochemical characterization and structural studies. The hABHD6 was the most promising variant without the first 29 amino acids and four aromatic residues (Y38F/V41) at the N-terminal end of the protein. It was expressed in BL21 E. coli cells under optimized conditions, and its activity was assessed using a fluorogenic and native substrates. The recombinant protein was successfully purified using immobilized metal affinity chromatography with or without detergent. The active site of the hABHD6 and the mode of interaction of selective inhibitors were characterized using ligand-assisted protein structure approach and matrix-assisted laser desorption/ionization time of flight mass spectrometry. The potent hABHD6 inhibitor AM11170 covalently interacted and selectively carbamoylated the enzyme active site serine residue. The stability of the hABHD6 protein alone and with inhibitors was evaluated in thermal shift assay to identify the most suitable samples for x-ray structural studies. Obtaining the crystal structure of hABHD6 will significantly improve the design of potent and selective ABHD6 inhibitors.

Introduction

The endocannabinoid system (ECS) plays a major regulatory role in various central and peripheral physiological processes of the human body. It consists of two cannabinoid receptors (CB1 & CB2), two endogenous ligands - anandamide and 2-AG, and enzymes that metabolize these ligands. In cells, the balance of 2-AG is regulated by several hydrolytic enzymes - MGL, ABHD6 and ABHD12 which control distinct subcellular pools of its degradation. ABHD6 accounts for 4% of 2-AG hydrolysis in the brain, and it is the main hydrolytic enzyme in other cell types. Therefore, ABHD6 becomes an attractive target for several therapeutic applications. 

Results

hABHD6 sequence and modifications that generated hΔ29-4W-ABHD6

- Enzyme activity and kinetic parameters were determined in both fluorescent and HPLC assays using the fluorogenic arachidonoyl, 7-hydroxy-6-methoxy-4-methylcoumarin ester (AHHMCE) and the native 2-arachidonyl glycerol (2-AG) substrates, respectively.
- Protein purifications were conducted using immobilized metal affinity chromatography (IMAC) through binding of the tagged protein to TALON resin.
- Mass spectrometric analysis of the tryptic digests of detergent-free purified hABHD6 treated or untreated with selective inhibitors were performed using matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) instrument.

Conclusions

The hΔ29-4W-ABHD6 variant was expressed, purified and characterized in biochemical and pharmacological assays. Using MALDI TOF MS analysis we identified that AM11170 inhibitor covalently modified the active site serine residue of ABHD6. Majority of evaluated inhibitors stabilized the ABHD6 protein as followed from thermal shift assays. The Tm of the enzyme-inhibitor complex was increased by 8 degrees in the presence of AM11197 or AM11943, indicating that these inhibitors are the most promising for x-ray structural studies of ABHD6.

References