Production and Characterization of fluorescent ATP Sensors with Enhanced pH Stability

Final Degree Project - Biochemistry and Molecular Biology
Confidential Academic Document

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AI-based language models were used during the writing process for minor linguistic refinement. No AI tools were involved in the generation, analysis, or interpretation of scientific content.

Summary

In synthetic biology, cell-like vesicles are engineered to replicate life-like functions such as protein expression, division, and metabolism within confined environments. These systems require maintenance of an ATP-fueled out-of-equilibrium state to sustain biochemical reactions. Precise, real-time quantification of ATP is critical for their functionality. However, current ATP sensing methods face significant limitations such as poor spatial-temporal resolution, sample destruction, i.e. no in-situ monitoring possible, and susceptibility to pH fluctuations. Genetically encoded biosensors have improved on some of these drawbacks, but often exhibit pH-dependent responses and measure ATP:ADP ratios rather than absolute ATP concentrations. In previous work, our group characterized PercevalHR, a genetically encoded sensor with good spatiotemporal resolution. However, its pH sensitivity and its inability to measure absolute ATP concentrations limits its application in complex systems like synthetic vesicles. We hypothesized that hybrid fluorophore-protein sensors based on the ε-subunit of the F_0F_1-ATP synthase from thermophilic *Bacillus* sp. PS3 can be developed and used to accurately detect ATP levels in real time, with improved stability under varying pH conditions.

We worked with two ATP sensors: a FRET-based sensor, bAT-1.03, which is a genetically encoded construct with two fused fluorescent proteins, and a hybrid fluorophore-protein sensor referred to as ATP-binding protein (ATPBP), which requires conjugation with fluorophores. Our primary objective was to purify both sensors, and in the case of the hybrid sensor, to label it with the dyes. Subsequently, we aimed to characterize their ATP-binding properties, fluorescence responses across varying pH conditions, and specificity for ATP over related nucleotides. Affinity chromatography followed by size exclusion chromatography were employed to purify the sensor variants from previously expressed proteins, with purification confirmed by SDS-PAGE analysis. Fluorescent labeling with dyes was performed by immobilizing the protein on a Ni-NTA resin column, and successful fluorophore conjugation was verified by spectrophotometry. Both sensors were characterized using plate reader measurements: bAT-1.03 demonstrated ATP-specific fluorescence response, pH stability, and nucleotide selectivity; whereas the ATPBP sensor exhibited effective discrimination across varying ATP concentrations. The results confirm our hypothesis: both ε-subunit-based ATP sensors detected and discriminated ATP. However, labeling was only partially successful, limiting further characterization of the labeled sensor. These findings offer new strategies for real-time ATP sensing in synthetic biology and highlight the need to test these sensors in vesicle-based, out-of-equilibrium environments to advance the development of life-like synthetic cells.

KEY WORDS: ATP sensor, fluorescent proteins, epsilon subunit, FRET sensor, pH-stable sensor