PRODUCTION AND FUNCTIONAL ANALYSIS OF CYSTEINE-MUTATED RECOMBINANT HUMAN MITOCHONDRIAL CARBONIC ANHYDRASES CAVA AND CAVB

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Carbonic anhydrases (CA) are ubiquitous enzymes that catalyze the reversible hydration of carbon dioxide into bicarbonate ($CO_2 + H_2O \rightleftharpoons H_2CO_3$).These enzymes play a vital role in physiological functions such as pH regulation and calcification. Among the fifteen human CAs, we focused on CA VA and CA VB, the only mitochondrial isozymes that provide bicarbonate ions during gluconeogenesis and lipogenesis[1].

The investigation of carbonic anhydrases is crucial for drug design due to their links with various pathological conditions, including cancer and obesity[2]. Our research aimed to produce recombinant proteins for human mitochondrial carbonic anhydrases CA VA and CA VB. In previous efforts to produce recombinant functional CA VA and CA VB, we encountered low solubility and aggregation challenges. To improve recombinant protein stability, we hypothesized that removing cysteine residues from the protein sequences would be beneficial. Using targeted mutagenesis, we replaced each cysteine with serine or alanine.

After completing the mutagenesis, we expressed the target proteins in *Escherichia coli* and purified them. We then evaluated the CO_2 hydratase activity of the recombinant CA VA and CA VB proteins by stopped-flow method and inhibitor binding using fluorescence thermal shift assay, as illustrated in Figure 1.

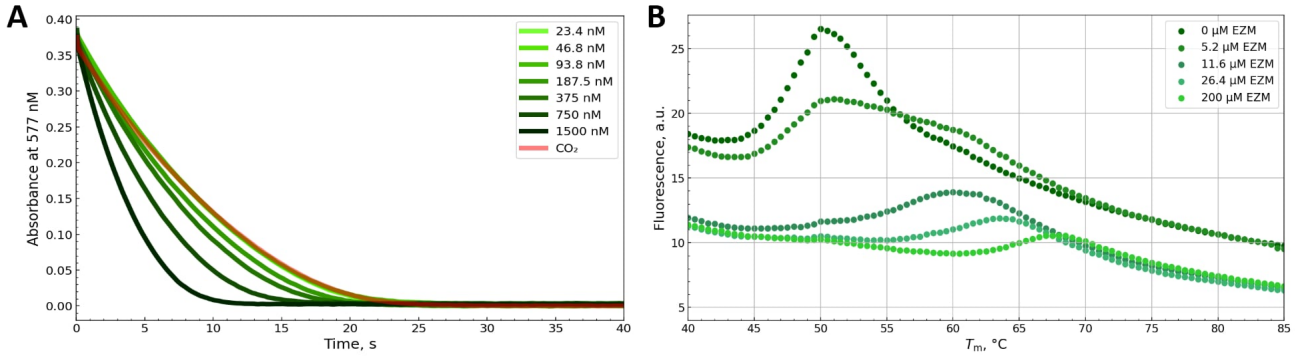

Fig. 1. A – CO_2 hydration activity of CA VB by stopped-flow analysis. Initial experimental curves for the determination of enzymatic activity, showing the changes in the absorption of the pH indicator over time depending on the concentration of CA VB, B – Ligand binding by fluorescence thermal shift assay: pure protein CA VB and protein with different concentrations of CA inhibitor ethoxzolamide (EZM).

[1] Aspatwar, A., Supuran, C.T., Waheed, A., Sly, W.S., Parkkila, S., 2023. Mitochondrial carbonic anhydrase VA and VB: properties and roles in health and disease. The Journal of Physiology 601, 257–274. https://doi.org/10.1113/JP283579

[2] Lomelino, C.L., Andring, J.T., McKenna, R., 2018. Crystallography and Its Impact on Carbonic Anhydrase Research. International Journal of Medicinal Chemistry 2018, 1–21. https://doi.org/10.1155/2018/9419521