Extraction and Optimization Experiments of the ABCA4 ECD1 Domain by Detergent and Affinity-Based Methods

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Purpose: The retina-specific ABCA transporter, ABCA4, is essential for vision, and its genetic variants are associated with a wide range of inherited retinal degenerative diseases leading to blindness, including Stargardt disease. The ABCA4 protein consists of characteristic functional domains: ECD1, ECD2, NBD1, and NBD2, alongside transmembrane domains (TMDs). ECD1 and ECD2 are positioned as extracytoplasmic domains of ABCA4.

The first extracellular domain of the ABCA4 ATP-binding cassette transporter is involved in retinoid binding, which drives the visual cycle. The target of the project is the expression and purification of its first extracytoplasmic domain (ECD1), for further structural and functional studies.

Methods: The study was focused on the extraction and purification of the ECD1 domain of ABCA4. Protein expression was induced in *E.coli* and confirmed by SDS-PAGE with Coomassie staining and Western blotting. The first phase of extraction was performed using commercial extraction buffers, followed by a second detergent extraction with FC-14 to obtain better recovery. The Bradford assay was used to measure protein concentrations before the SDS-PAGE analysis. Nickel affinity chromatography was performed to further purify the ECD1 domain.

Results: SDS-PAGE and Western blot analysis confirmed ECD1 expression following induction. Initial extraction produced a low yield of soluble ECD1, while FC-14 detergent extraction displayed a significant improvement in solubility. Nickel affinity purification yielded ECD1 protein fractions with enhanced purity.

Conclusions: Successful expression and preliminary recovery of the ABCA4 ECD1 domain are a significant step towards its biochemical characterization. Optimal purification of the domain is critical since it will facilitate extensive retinoid-binding assays and structural studies. Defining the function of this domain will inform how specific ECD1 mutations disrupt retinoid interactions and cause retinal degenerative disease. This work lays the groundwork for further investigation of the ECD1 variant pathogenicity and paves the way to direct therapeutic strategies in ABCA4-associated disorders.