EXPRESSION OF A MEMBRANE TRANSPORTER – SCALE-UP, STABILITY, CONTAMINATION PROFILE, AND CRYSTALLIZATION TRIALS

Gerda Szakonyi[1], Emese Zsuzsanna Galgóczi[1], Lóránt Lakatos[2]

[1]University of Szeged, Faculty of Pharmacy, Institute of Pharmaceutical Analysis, H-6720 Szeged, Somogyi u. 4.,
[2]Lóránt Eötvös Research Network, Biological Research Center, Institute of Plant Biology
e-mail: szakonyi.gerda@szte.hu

Among the methods used to study the structure of membrane proteins, X-ray crystallography can be considered the most frequently used method. However, the crystallization often fails; therefore, a so-called chaperone protein was fused to the target protein to promote crystallization.

During our work, we coupled the fucose transporter of *Haemophilus influenzae* HI0610 with the T4 lysozyme chaperone protein. Gel electrophoresis confirmed that the desired fusion protein was expressed.

BL21(DE3) *E. coli* cells were grown in 2XY, LB, and autoinduction medium to optimize the fusion protein expression. Compared to IPTG-induced samples, the protein produced by autoinduction gave us the most satisfying result. After optimization of the expression conditions, we produced the fusion protein in larger quantities, thus ensuring the production of a sufficient amount of target molecule for crystallization.

The fusion protein was purified further by affinity, size exclusion, and ion exchange chromatography to high prurity.

The purified recombinant protein was then used to determine the secondary structure by circular dichroism. The CD spectrum of the fusion protein mainly showed alpha-helical and parallel beta-sheet secondary structures, which is in agreement with the alpha helical structure of the transporter protein and the beta-sheet N-domain of the T4 lyzozyme. Also, the protein was highly resistant to trypsin treatment, which ensures its stability.

Finally, using the hanging drop technique, we tested more than 300 crystallization conditions resulting in the presence of microcrystals. The observed microcrystals are promising signs for further structural studies; however, additional optimization is necessary.

In the future, we would like to determine the conditions for the transporter's crystallization and understand the protein's structure and stability using other analytical tools.