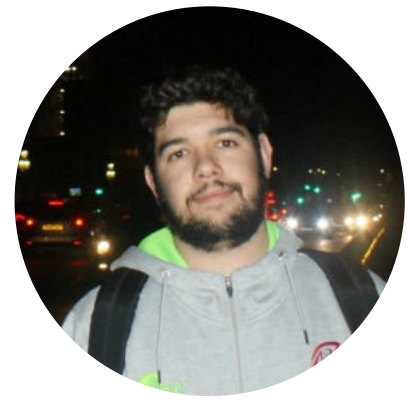
Cannabidiol effect on α-synuclein solution behaviour



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INTRODUCTION

Parkinson's Disease (PD) is a fast-growing disease, especially in the last generation. It is known that some chemicals, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and amphetamine-type stimulants are related to PD onset. The neuronal protein α-synuclein aggregation, is the hallmark of PD. This protein can be a pathological condition when overexpressed and/or modified, leading to α -synuclein neurotoxic aggregates [1, 2, 3] . Cannabis is used by PD patients worldwide to reduce symptoms. However, phytocannabinoids medical use has not been approved for PD. Recent studies suggest that the effects of phytocannabinoids are mediated by CB1 and/or CB2 receptors

This experiment was conducted with the objective of better understanding the interactions between alpha-synuclein and phytocannabinoids, by studying α –synuclein's behaviour in solution when exposed to Cannabidiol (CBD).

E. coli was used in this study to express α-synuclein and published conditions were used for purification[4]. After purification, α-synuclein was incubated with CBD. Aliquots collected at different time stamps were used to analyse the concentration of the protein, secondary structure conformation and aggregation.

EXPERIMENTAL PROCEDURE

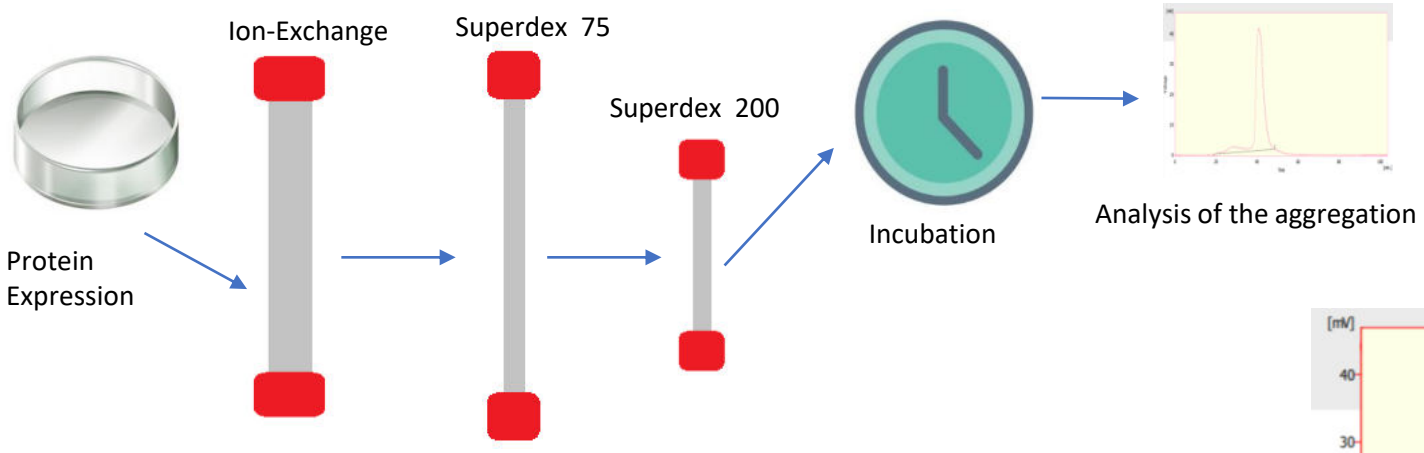

Figure 1: Experimental procedure carried out in this experiment.

RESULTS

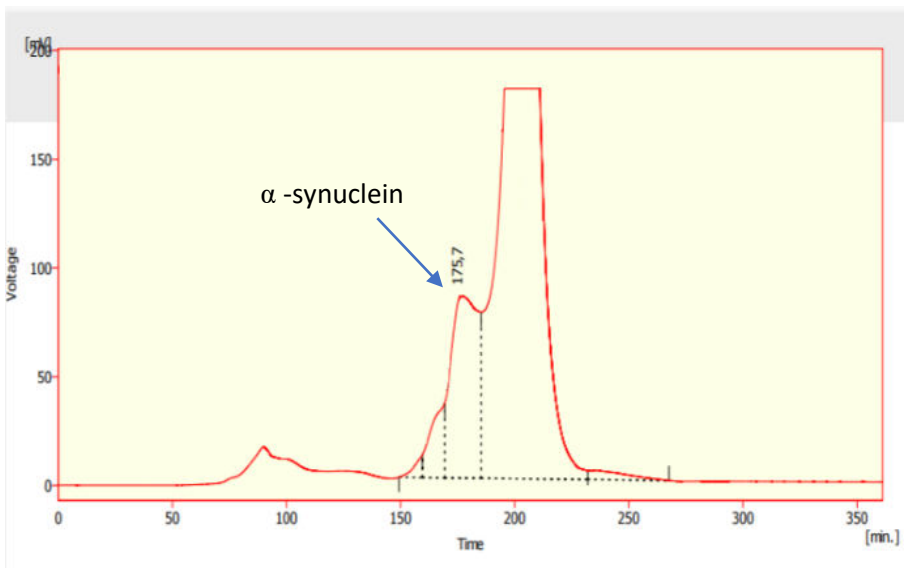

Figure 2: Purification of α -synuclein through Ion-Exchange Chromatography

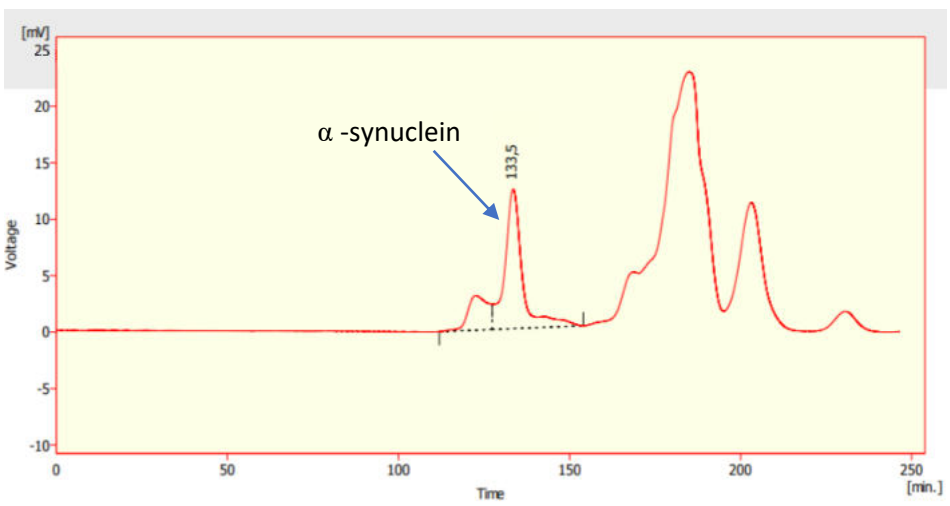

Figure 3: Purification of α –synuclein through Size-Exclusion Chromatography (Superdex 75)

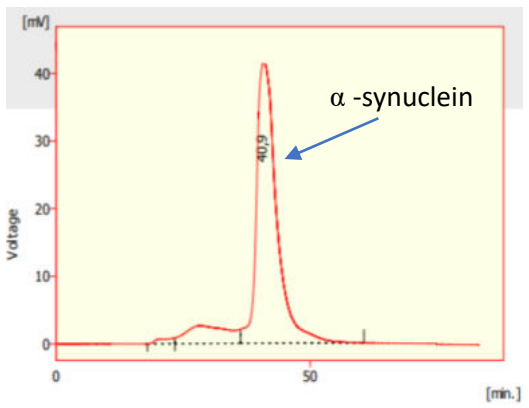

Figure 4: Purification of α –synuclein through Size-Exclusion Chromatography (Superdex 200)

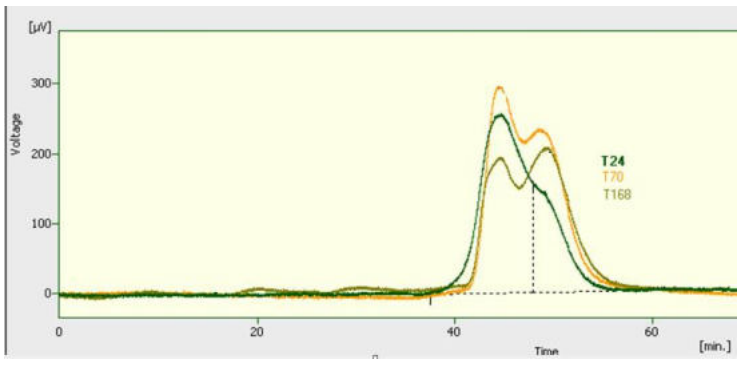

Figure 5: Variation of the proportion of the oligomeric species after 24, 70 and 168 hours of incubation, being the retention times of 44 and 49 minutes, corresponding to tetramers and dimers.

CONCLUSIONS

This experiment allowed for:

- The optimization of the expression and purification of α-synuclein;
- The observation of several soluble oligomeric species of α-synuclein;
- Observation of changes in the proportions of the oligomeric species in the presence of CBD;

A future experiment with different concentrations of α-synuclein, could be performed in order to study its impact on aggregation.

[1] - Wegrzynowicz, M., Bar-On, D., Calo', L., Anichtchik, O., Iovino, M., & Xia, J. et al. (2019). Depopulation of dense α-synuclein aggregates is associated with rescue of dopamine neuron dysfunction and death in a new Parkinson's disease model. Acta Neuropathologica, 138(4), 575- 595. doi: 10.1007/s00401-019-02023-x.
[2] - Cookson, M. (2009). α-Synuclein and neuronal cell death. Molecular Neurodegeneration, 4(1), 9. doi: 10.1186/1750-1326-4-9
[3] - Yasuda, T., Nakata, Y., & Mochizuki, H. (2012). α-Synuclein and Neuronal Cell Death. Molecular Neurobiology, 47(2), 466-483. doi: 10.1007/s12035-012-8327-0
[4] - Outeiro, T., Klucken, J., Bercury, K., Tetzlaff, J., Putcha, P., & Oliveira, L. et al. (2009). Dopamine-Induced Conformational Changes in Alpha-Synuclein. Plos ONE, 4(9), e6906. doi: 10.1371/journal.pone.0006906