ORT_13 - Expression, purification and characterization of $S_{542-931}$ and $RBD_{330-524}$ from spike protein of SARS-Cov-2

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Introduction: SARS-CoV-2 is an enveloped, non-segmented, positive sense RNA virus and the viral genome encodes non-structural and structural proteins. One of these structural proteins is the spike glycoprotein (S) that is N-glycosylated and trimers of this protein peak are found in the surface structure of the virus. This protein is cleaved by a host protease into two functional domains with the S1 containing the RBD - Receptor Binding Domain - being responsible for binding virus to the human cell receptor. The S1 protein has been used as a potential target for diagnostic tests and vaccine development.

Objective: To express, purify and characterize the spike fragments $S_{542-931}$ and $RBD_{330-524}$ from SARS-Cov-2 (GenBank: MN988669.1) to be used in vaccines and diagnostic tests.

Methodology: The genetic constructions were acquired from the companies (GenScript and Biomatik) and transformed into *E. coli* strains. The protein expression was performed at 28ºC and 37ºC in LB medium and the expression and identity were determined by SDS-PAGE and *western blotting*. For both proteins the inclusion bodies were washed out and extracted using urea. Protein purifications were performed using IMAC chromatography in FPLC equipment and the refolding were performed by slow dilution using the dropwise method. Purified proteins were analyzed by size exclusion chromatography (SEC), gradient SDS-PAGE (4-12%) and IEF-PAGE (3.0–9.0). Tryptophan fluorescence emission spectra was obtained by excitation wavelength at 280 nm and the emission spectra was recorded from 295 to 415 nm. Circular Dichroism (CD) spectra was monitored from 190 to 260 nm and the kinetic thermal denaturation of $S_{542-931}$ protein was determined in a temperature range (25ºC–85ºC).

Results: Despite the use of different strains of *E. coli* to express the proteins, different induction temperatures and use of additives in the lysis buffer, the recombinant proteins were expressed in inclusion bodies. The proteins were purified with a high level of purity and refolded using some additives. The $S_{542-931}$ protein showed several bands in SDS-PAGE, but in presence of reducing agent presented a single band with 43 kDa (99.8% homogeneity). SEC analysis results were similar to SDS-PAGE, showing a complex profile with four protein peaks. IEF-PAGE demonstrated isoelectric point of 6.2 to $S_{542-931}$ protein. The tryptophan fluorescence and CD thermogram showed a progressive decay with the increase of temperature and the CD spectrum of $S_{542-931}$ protein structure demonstrated random coil 44.5% of alpha helix and 55.5% of beta-sheet. The $RBD_{330-524}$ has already been purified with a purity greater than 90% and we are currently working on protein refolding and characterization.

Conclusion: The proteins were successfully expressed, purified and characterized. These proteins are being used in different approaches like diagnosis and vaccine development.

Keywords: SARS-CoV-2; spike protein; RBD protein