ORT_01 - Investigation of recombinant SARS-CoV-2 nucleocapsid protein thermal properties and its nucleic acid interaction

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Introduction: The role of nucleocapsid (N) protein from SARS-Cov-2 is not only to compose viral particle, but also contributes to many critical activities after virus invasion. Functional diversity is intimately associated to dynamic structure and its ability to bind and change RNA structure. Knowledge of protein properties on native fold allows several applications from drug design to the optimization of its activity.

Objectives: This study investigated recombinant SARS-CoV-2 N protein thermal properties and its nucleic acid interaction.

Methodology: Benzonase was used on nucleic acid remotion. Molecular weight was evaluated by SEC-MALS. Investigation of thermal properties were performed by circular dichroism spectroscopy (CD), fluorescence spectroscopy, nano-differential scanning fluorimetry (NanoDSF) and microscale thermophoresis (MST).

Results: N protein nucleic acid associated (NA) revealed as tetramer form (171.2 kDa) and N protein benzonase treated (NB) as dimer form (104.8 kDa). NanoDSF revealed 2 transitions and only the first had similar unfolding melting temperature (T_m^1) of 46.13 ±0.38°C and 45.36 ±0.03°C for NA and NB, respectively, and T_m^2 showed differences (73.49 ±0.41oC for NA and 76.80 ± 0.18°C for NB). Both proteins restored secondary and tertiary structures after thermal kinetic, demonstrating thermal stability up to 6 cycles. Tertiary structural analysis with different NA and NB concentrations revealed alteration only at T_m^2. CD experiments corroborated these results indicating a single transition between 40-50°C, independent of NA or NB concentrations. Data showed T_m^1 is related to protein denaturation and T_m^2 to oligomers dissociation suggesting nucleic acid remotion promotes higher stability in homodimer structure. MST analysis indicated higher affinity of anti-N protein monoclonal antibody for NB (Kd 157.3 ± 19 nM) than NA (Kd 274 ± 63 nM). Renatured-NB showed similar affinity (Kd141.2 ± 9 nM) to native one, while renatured-NA showed lower affinity (Kd 424.6 ± 34 nM).

Conclusion: Results showed NA and NB have similar denaturation temperatures, independently of nucleic acid presence. Both were able to restore secondary and tertiary structures after thermal kinetic. We demonstrated the interference of nucleic acid in molecular affinity of N protein.

Keywords: COVID-19; Thermal stability; Structural properties