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Effects of the SARS-CoV-2 Spike protein on in vitro aggregation of alpha synuclein- probable molecular interactions and clinical implications

Sankha Shubhra Chakrabarti[1], Shagun Gupta[2], Upasana Ganguly[2], Ankur Kaushal[2], Ashish Kumar Yadav[1], Upinder Kaur, Adesh Kumar Saini[2], Sasanka Chakrabarti[2]

1 Banaras Hindu University

2 Maharishi Markandeshwar University, Mullana

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Abstract

There have been reports of neurodegenerative diseases including Parkinsonism, Alzheimer's disease and Creutzfeldt Jakob disease following COVID-19. The exact pathogenesis of these has not been elucidated yet though authors have proposed the possibility of the hyper-inflammatory state of COVID-19 acting as a trigger through cytokine-induced inflammatory response of brain microglia and astrocytes, as well as damage resulting from the central nervous system hypoxia of severe COVID-19, or even the direct pathogenetic actions of SARS-CoV-2 on the brain. There have also been reports of worsening of Parkinsonian symptoms, new onset movement disorders and even rapidly progressive dementia following COVID-19 vaccination using different vaccine types. The occurrence of protein-aggregation mediated neurodegenerative syndromes following both COVID-19 and vaccination led us to explore the common thread of the Spike (S) protein as a potential mediator. In the current study, we investigated the interactions of S protein of SARS-CoV-2 with α-synuclein.

Sankha Shubhra Chakrabarti[1], **Shagun Gupta**[2], **Upasana Ganguly**[3], **Ankur Kaushal**[2], **Ashish Kumar Yadav**[4], **Upinder Kaur**[5], **Adesh Kumar Saini**[2], **Sasanka Chakrabarti**[3*]

[1] *Department of Geriatric Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India*

[2] *Department of Biotechnology and Central Research Cell, Maharishi Markandeshwar (deemed to be) University, Mullana, Ambala*

[3] *Department of Biochemistry and Central Research Cell, Maharishi Markandeshwar (deemed to be) University, Mullana, Ambala*

[4] *Center for Biostatistics, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India*

[5] *Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India*

*Corresponding author: Prof Sasanka Chakrabarti, Department of Biochemistry and Central Research Cell, Maharishi Markandeshwar (deemed to be) University, Mullana, Ambala, Haryana, India, PIN-133203;
Email: profschakrabarti95@gmail.com; Phone: +919874489805

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1. Introduction

There have been reports of neurodegenerative diseases including Parkinsonism, Alzheimer's disease and Creutzfeldt Jakob disease following COVID-19.[1][2][3] The exact pathogenesis of these has not been elucidated yet. Authors of several isolated case reports as well as reviews have suggested the possibility of the hyper-inflammatory state of COVID-19 acting as a trigger through cytokine-induced inflammatory response of brain microglia and astrocytes, as well as damage resulting from the central nervous system hypoxia of severe COVID-19, or even the direct pathogenetic actions of SARS-CoV-2 on the brain.[1][2][3] Similarly, there have been reports of worsening of Parkinsonian symptoms, new onset movement disorders and even rapidly progressive dementia following COVID-19 vaccination using different vaccine types, including one from our group.[4][5] The occurrence of protein-aggregation mediated neurodegenerative syndromes following both COVID-19 and vaccination led us to explore the common thread of the Spike (S) protein as a potential mediator. In the current study, we were interested particularly to investigate the interactions of S protein of SARS-CoV-2 with α-synuclein in order to understand the molecular links of COVID-19 and Parkinsonian pathogenesis.

2. Materials and Methods

2.1. Docking Studies

In the first step, we performed protein–protein docking using the software HDOCK which is an integrated suite of homology search, template-based modeling, structure prediction, macromolecular docking, biological information incorporation and job management for protein–protein docking.[6] The server automatically predicts the interaction of receptor and ligand molecules (amino acid sequences or Protein Data Bank, PDB structures), through a hybrid algorithm of template-based and template-free docking. For the current study, the PDB structures PDB: 1XQ8, and 6VXX for α-synuclein, and SARS-CoV-2 Spike protein, respectively, were downloaded and used for docking.

2.2. *In vitro* experiments

We further evaluated whether the predicted interactions of S-protein and α-synuclein actually altered the aggregation

process of the latter under *in vitro* conditions. We incubated α-synuclein (10 μM) in 50 mM phosphate buffer, at pH 7.4 at 37^0C for 120 h with 10 μM S-protein (group 1) or without S-protein (group 2); separately S-protein (10 μM) alone was also incubated under identical conditions (group 3). All groups had 4 samples each. Aliquots were removed from the incubation mixtures (all groups) at definite 24-hourly intervals, and the oligomerization of α-synuclein *in vitro* was measured spectrofluorometrically using the Thioflavin T assay as adopted from a published paper.[7] The thioflavin-T assay is a good method to examine the oligomerization or aggregation of proteins like amyloid β42 and α-synuclein; a strong fluorescence is emitted when thioflavin-T binds to the oligomerized or aggregated forms of these proteins.

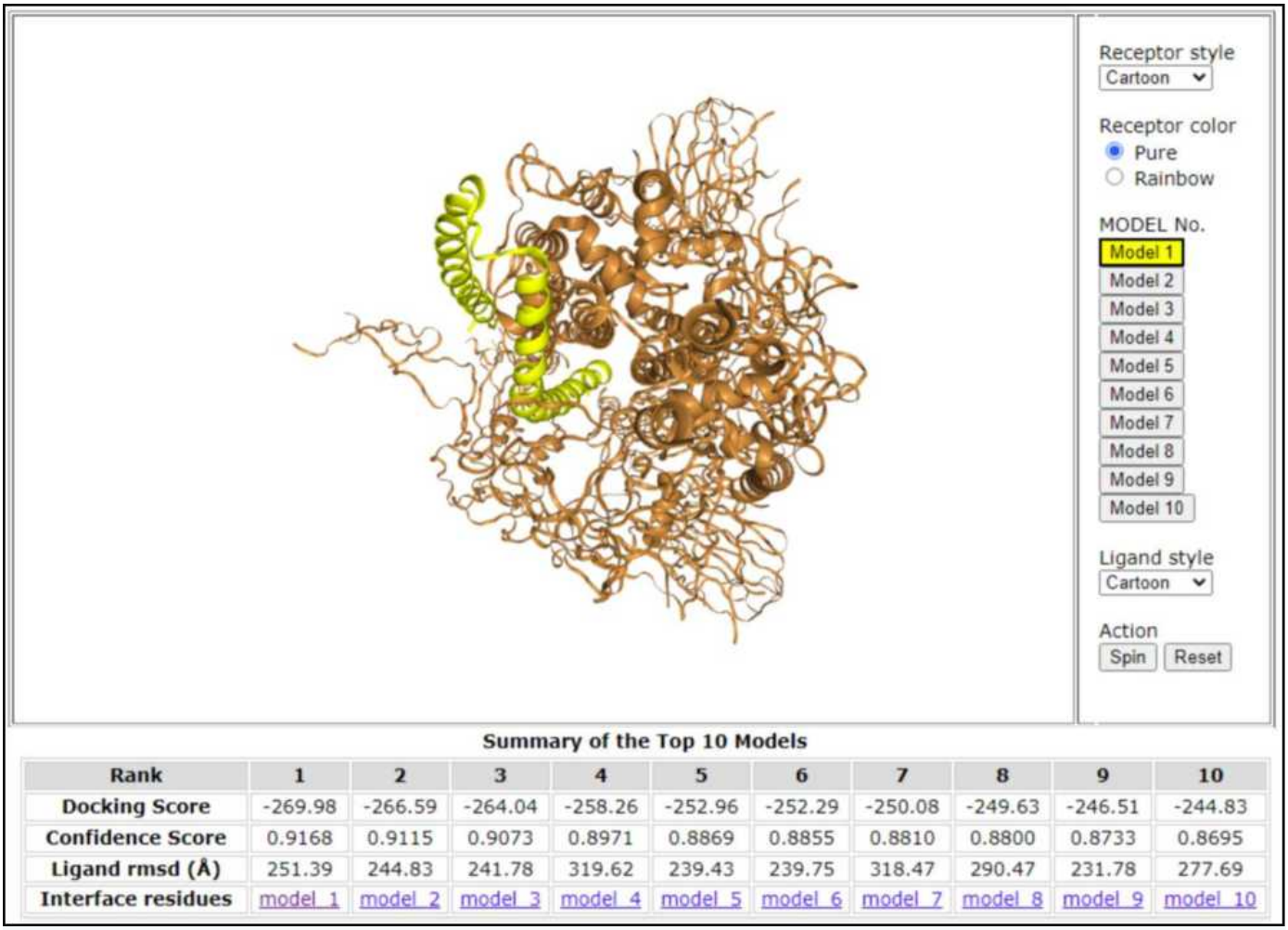
2.3. Statistical Analysis

To determine statistical significance of variations in α-synuclein aggregation with time between the groups 1,2, and 3 a DOE (design of experiment) model was used. The variation in the three groups over six days duration was evaluated using a factorial experiment with repeated measures ANOVA.

3. Results

3.1. Docking Studies

Around 20 docked models were generated out of which model 1 was selected based on the lowest docking energy score (-269.98). The ligand root mean square deviation (RMSD) was 251.39 Å. The confidence score was also found to be above 0.9 indicating the binding likeliness of the two molecules as depicted in **Figure 1**. The interface residues within 5.0 Å from their interacting partner and the corresponding distances in the model are shown in the **Supplementary File**.



Summary of the Top 10 Models

Rank	1	2	3	4	5	6	7	8	9	10
Docking Score	-269.98	-266.59	-264.04	-258.26	-252.96	-252.29	-250.08	-249.63	-246.51	-244.83
Confidence Score	0.9168	0.9115	0.9073	0.8971	0.8869	0.8855	0.8810	0.8800	0.8733	0.8695
Ligand rmsd (Å)	251.39	244.83	241.78	319.62	239.43	239.75	318.47	290.47	231.78	277.69
Interface residues	model_1	model_2	model_3	model_4	model_5	model_6	model_7	model_8	model_9	model_10

Figure 1. A docking summary of the top 10 models generated by HDOCK. *The docking score, confidence score and ligand rmsd (in Å) are provided for the top 10 models. The interaction between the two proteins for model 1 is represented pictorially. Yellow- α-Synuclein; Brown- Spike protein.*

3.2. *In vitro* experiments

We noticed a distinct lag phase in the aggregation process of α-synuclein (group 2). Interestingly, in the presence of S protein, the lag phase was abolished and a more rapid onset of α-synuclein aggregation took place (group 1). However, the extent of α-synuclein aggregation in groups 1 and 2 eventually became similar with time (**Figure 2**). Further, S protein by itself failed to show any aggregation as measurable by thioflavin-T assay (group 3). The trend lines in **Figure 2** differed between the three groups. These findings were supported by ANOVA results where significant variations ($P < 0.000$) in α-synuclein aggregation with time was observed between the groups 1, 2, and 3 (**Supplementary File**).

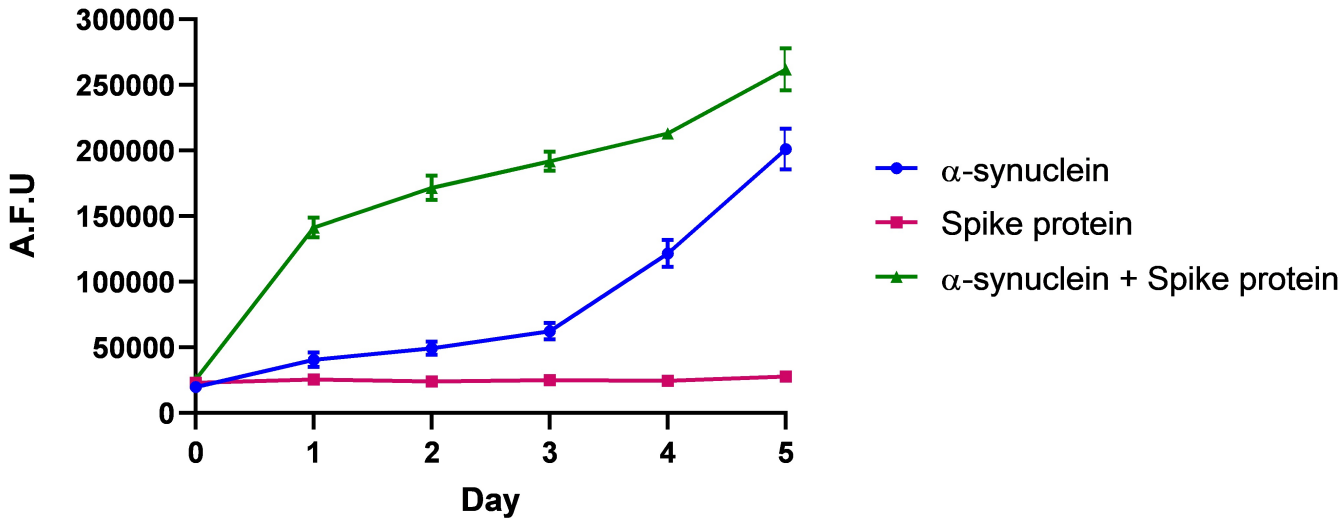


Figure 2. Comparison of the aggregation rates in the three groups as measured spectrofluorometrically using the Thioflavin T assay. Group 1- green line (α-Syn + Spike protein); Group 2- blue line (α-Syn); Group 3- pink line (Spike protein). Abbreviation: A.F.U: Arbitrary fluorescence unit.

4. Discussion

Previous studies using the aggregation-prediction server- AGGRESCAN and protein-protein docking software HDOCK (http://hdock.phys.hust.edu.cn/) indicated that the S1 subunit of the S-protein of SARS-CoV-2 can interact with other aggregation-prone proteins of the brain like amyloid beta, prion protein, α-synuclein, and tau, presumably through heparin-binding domains, to form homo or hetero-polymers resembling amyloid fibrils. These may play a causal role in the neurodegenerative process of the misfolded protein disorders.[8][9] Similar observations were made by us.

We further explored possible effects of the S-protein on α-synuclein aggregation. The lag period observed by us in group 2 (α-synuclein alone) is a well-known phenomenon of α-synuclein aggregation *in vitro*, and this has been attributed to the nucleation-dependent aggregation of α-synuclein.[10] Since this lag period disappeared in group 1 (α-synuclein with Spike), it is tempting to hypothesize that initial interactions of α-synuclein and S protein, as predicted by protein-protein docking studies, created many nucleation centers which accelerated the process of α-synuclein aggregation.

Earlier, an elaborate study from China demonstrated that the S and N proteins of SARS-CoV-2 could upregulate the expression of -synuclein as well as its aggregation in HEK293 cells in culture.[11] Another study reported that N protein but not S protein of SARS-CoV-2 enhanced α-synuclein aggregation; this study used thioflavin-T assay, but used a concentration of S protein 10 to 100 times lower than that used by us.[12] Taken together these studies including ours indicate some probable links between Parkinson's disease and COVID-19 through α-synuclein aggregation. Our experiments showing an early enhancement of α-synuclein aggregation by S protein may imply that in persons at an early stage of Parkinson's disease without discernible motor disability, the disease process may be hastened up following

exposure to the S protein from SARS-CoV-2. There also exists the possibility of such rare events occurring on exposure to S protein generated by COVID-19 vaccination strategies based on the mRNA or Adenoviral platforms which trigger its production in the human body. Both increased vigilance and further research may be warranted for post-COVID or post-vaccination neurological effects.

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References

1. [a, b]*Bernardini, A.; Gigli, G.L.; Janes, F.; Pellitteri, G.; Ciardi, C.; Fabris, M.; Valente, M. Creutzfeldt-Jakob Disease after COVID-19: Infection-Induced Prion Protein Misfolding? A Case Report. Prion 2022, 16, 78–83, doi:10.1080/19336896.2022.2095185.*
2. [a, b]*Boura, I.; Chaudhuri, K.R. Coronavirus Disease 2019 and Related Parkinsonism: The Clinical Evidence Thus Far. Mov. Disord. Clin. Pract. 2022, 9, 584–593, doi:10.1002/mdc3.13461.*
3. [a, b]*Matias-Guiu, J.A.; Delgado-Alonso, C.; Yus, M.; Polidura, C.; Gómez-Ruiz, N.; Valles-Salgado, M.; Ortega-Madueño, I.; Cabrera-Martín, M.N.; Matias-Guiu, J. "Brain Fog" by COVID-19 or Alzheimer's Disease? A Case Report. Front. Psychol. 2021, 12, doi:10.3389/fpsyg.2021.724022.*
4. [^]*Imbalzano, G.; Ledda, C.; Artusi, C.A.; Romagnolo, A.; Montanaro, E.; Rizzone, M.G.; Lopiano, L.; Zibetti, M. SARS-CoV-2 Vaccination, Parkinson's Disease, and Other Movement Disorders: Case Series and Short Literature Review. Neurol. Sci. 2022, 43, 5165–5168, doi:10.1007/s10072-022-06182-w.*
5. [^]*Chakrabarti, S.S.; Tiwari, A.; Jaiswal, S.; Kaur, U.; Kumar, I.; Mittal, A.; Singh, A.; Jin, K.; Chakrabarti, S. Rapidly Progressive Dementia with Asymmetric Rigidity Following ChAdOx1 NCoV-19 Vaccination. Aging Dis. 2022, 13, 633, doi:10.14336/AD.2021.1102.*
6. [^]*Yan, Y.; Tao, H.; He, J.; Huang, S.-Y. The HDOCK Server for Integrated Protein–Protein Docking. Nat. Protoc. 2020, 15, 1829–1852, doi:10.1038/s41596-020-0312-x.*

7. ^*Sinha, M.; Bhowmick, P.; Banerjee, A.; Chakrabarti, S. Antioxidant Role of Amyloid β Protein in Cell-Free and Biological Systems: Implication for the Pathogenesis of Alzheimerdisease. Free Radic. Biol. Med. 2013, 56, 184–192, doi:10.1016/j.freeradbiomed.2012.09.036.*
8. ^*Tavassoly, O.; Safavi, F.; Tavassoly, I. Seeding Brain Protein Aggregation by SARS-CoV-2 as a Possible Long-Term Complication of COVID-19 Infection. ACS Chem. Neurosci. 2020, 11, 3704–3706, doi:10.1021/acschemneuro.0c00676.*
9. ^*Idrees, D.; Kumar, V. SARS-CoV-2 Spike Protein Interactions with Amyloidogenic Proteins: Potential Clues to Neurodegeneration. Biochem. Biophys. Res. Commun. 2021, 554, 94–98, doi:10.1016/j.bbrc.2021.03.100.*
10. ^*Wood, S.J.; Wypych, J.; Steavenson, S.; Louis, J.-C.; Citron, M.; Biere, A.L. α-Synuclein Fibrillogenesis Is Nucleation-Dependent. J. Biol. Chem. 1999, 274, 19509–19512, doi:10.1074/jbc.274.28.19509.*
11. ^*Wu, Z.; Zhang, X.; Huang, Z.; Ma, K. SARS-CoV-2 Proteins Interact with Alpha Synuclein and Induce Lewy Body-like Pathology In Vitro. Int. J. Mol. Sci. 2022, 23, 3394, doi:10.3390/ijms23063394.*
12. ^*Semerdzhiev, S.A.; Fakhree, M.A.A.; Segers-Nolten, I.; Blum, C.; Claessens, M.M.A.E. Interactions between SARS-CoV-2 N-Protein and α-Synuclein Accelerate Amyloid Formation. ACS Chem. Neurosci. 2022, 13, 143–150, doi:10.1021/acschemneuro.1c00666.*