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Conformational flexibility and structural variability of SARS-CoV2 S protein

Ishika Pramanick,[1,3] Nayanika Sengupta,[1,3] Suman Mishra,[1] Suman Pandey,[2] Nidhi Girish,[2] Alakta Das,[1] and Somnath Dutta[1,4,*]

[1]Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, India
[2]Mynvax Private Limited, ES12, Entrepreneurship Centre, SID, Indian Institute of Science, Bengaluru, India
[3]These authors contributed equally
[4]Lead contact
*Correspondence: somnath@iisc.ac.in
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SUMMARY

Spike (S) glycoprotein of SARS-CoV2 exists chiefly in two conformations, open and closed. Most previous structural studies on S protein have been conducted at pH 8.0, but knowledge of the conformational propensities under both physiological and endosomal pH conditions is important to inform vaccine development. Our current study employed single-particle cryoelectron microscopy to visualize multiple states of open and closed conformations of S protein at physiological pH 7.4 and near-physiological pH 6.5 and pH 8.0. Propensities of open and closed conformations were found to differ with pH changes, whereby around 68% of S protein exists in open conformation at pH 7.4. Furthermore, we noticed a continuous movement in the N-terminal domain, receptor-binding domain (RBD), S2 domain, and stalk domain of S protein conformations at various pH values. Several key residues involving RBD-neutralizing epitopes are differentially exposed in each conformation. This study will assist in developing novel therapeutic measures against SARS-CoV2.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), belonging to the Coronaviridae family, is the third known zoonotic virus to have plagued mankind in the 21st century (Drosten et al., 2003; Huang et al., 2020; Zaki et al., 2012; Zhou et al., 2020b). This formerly unexplored coronavirus was first isolated from Hubei province, China, in December 2019 (Zhou et al., 2020a). As of November 1, 2020, the World Health Organization declared nearly 46.6 million infections and ~1.2 million deaths worldwide (WHO Daily Report). Since the release of the complete sequence of the SARS-CoV2 genome, a plethora of studies have been performed in search of possible therapeutic and vaccine candidates. One such thoroughly investigated vaccine generation target is the heavily glycosylated homotrimeric Spike (S) protein responsible for the crown-like surface display in these so-called coronaviruses (Tortorici and Veesler, 2019). Among the three transmembrane envelope proteins, S protein is known to mediate viral entry into host cells. The S protein in its prefusion state has two distinct subunits called S1 and S2. S1 harbors the receptor-binding domain (RBD), N-terminal domain (NTD), and two small subunits SD1 and SD2, while the S2 subunit has three stable long helices, which tether the S1 domain as well as the S protein with the viral envelope. Early studies have shown that this recently emerged coronavirus binds tightly to human ACE2 (hACE2) receptor, thereby facilitating its transmission (Walls et al., 2020). Specifically, the RBD of the S protein exists in two prominent conformations, up (open) and down (closed) (Toelzer et al., 2020; Walls et al., 2020; Wrapp et al., 2020; Xiong et al., 2020). These surface proteins exist as dimorphic entities before and after the fusion of viral and cellular membranes. Following membrane fusion, the S protein is cleaved by host cell proteases at the boundary between the S1 and S2 subunits, transforming into an elongated post-fusion state (Kirchdoerfer et al., 2016). Thus, owing to a pivotal role of S protein in eliciting the infection cascade, it is the most well-characterized viral structural protein and is widely used to isolate neutralizing antibodies (Du et al., 2009; Elshabrawy et al., 2012; Jiang et al., 2014; Li et al., 2015; Wang et al., 2015; Ying et al., 2015; Yu et al., 2015). A large number of structural studies indicate appreciable flexibility of the RBD region and unequivocally report the presence of the distinct 1-RBD up-open and all-RBD down-closed species of S protein trimers (Ke et al., 2020; Korber et al., 2020; Melero et al., 2020; Walls et al., 2020).

Unless stabilized by mutations, purified soluble S protein trimers have not been observed to display a 2-RBD up-open or all-RBD up-open conformation that could possibly lead to a more lethal SARS-CoV2 infection (Korber et al., 2020). Cryoelectron tomography (cryo-ET) analysis of intact virions displaying S proteins affirms past knowledge and reports a minor 14% population in 2-RBD up-open state (Ke et al., 2020). The envelope proximal stalk of the S2 subunit was recently investigated in a membrane-bound state and was found to act as a hinge around which the S trimer is free to rotate (Ke et al., 2020; Turoňová et al., 2020). Extensive characterization of MERS-CoV and SARS-CoV show a moderately flexible NTD, although the most dramatic