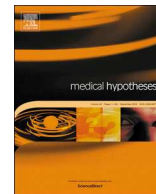




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Unique fibrinogen-binding motifs in the nucleocapsid phosphoprotein of SARS CoV-2: Potential implications in host-pathogen interactions

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ABSTRACT

Novel Coronavirus (SARS CoV-2), the etiological agent for the highly contagious Corona virus disease-2019 (COVID-19) pandemic has threatened global health and economy infecting around 5.8 million people and causing over 359,200 deaths (as of 28th May 2020, <https://www.worldometers.info/coronavirus/>). The clinical manifestations of infected patients generally range from asymptomatic or mild to severe illness, or even death. The ability of the virus to evade the host immune response have been major reasons for high morbidity and mortality. One of the important clinical observations under conditions of critical illness show increased risk of developing disseminated intravascular coagulation. Molecular mechanisms of how SARS CoV-2 induces such conditions still remain unclear. This report describes the presence of two unique motifs in the SARS CoV-2 nucleocapsid phosphoprotein (N-protein) that can potentially interact with fibrinogen and possibly prothrombin. This is based on an established function of secretory proteins in *Staphylococcus aureus* (*S. aureus*)-coagulase, Efb (Extracellular fibrinogen binding) and vWBP (von Willebrand factor Binding Protein), which are known to regulate the blood clotting cascade and the functions of host immune response. It is hypothesized that having protein interaction motifs that are homologous to these *S. aureus* proteins, the N-protein of this virus can mimic their functions, which may in turn play a crucial role in formation of blood clots in the host and help the virus evade host immune response. However, this hypothesis needs to be tested *in vitro*. Considering the overwhelming increase in the incidence of SARS CoV-2 infection globally, this information may be useful for further investigation and could help in deducing new therapeutic strategies to combat advanced stages of this disease.

Introduction

The Coronavirus disease-2019 (COVID-19) pandemic outbreak has caused devastating consequences in the lives of people globally. Intensive investigations are underway to understand the molecular mechanisms that SARS CoV-2 adopts for its survival in the host. Coagulation disorders have been recently reported in SARS CoV-2 infected patients [1]. High levels of D-dimer clearly indicated excessive activation of coagulation cascade in patients [2]. In addition to this, the ability of the virus to evade immune response has made it very difficult for clinicians to treat this disease. Therefore, it is important to understand the molecular adaptations of SARS CoV-2 which are crucial for host-pathogen interactions and host immune evasion strategies [3]. This article presents a hypothesis based on identification unique fibrinogen-interaction motifs and possible prothrombin-interaction region on the nucleocapsid phosphoprotein (N-protein) that is homologous to three secretory proteins in *Staphylococcus aureus* (*S. aureus*)

coagulase, Efb (Extracellular fibrinogen binding) and vWBP (von Willebrand factor Binding Protein). It is hypothesized that the presence of these motifs on N-protein would enable it to mimic the functions of the three proteins of *S. aureus* which are well known to activate the coagulation cascade in the host and ensure pathogen survival by steering clear of host immunity by regulating other important signaling pathways like opsonization and phagocytosis.

Medical hypothesis

The N-protein of the Coronavirus family is a multifunctional protein which is primarily involved in packaging of the RNA genome and anchoring the ribonucleoprotein to the viral membrane via its interaction with the (matrix) M protein [4]. In quest of finding important motifs in the N-protein of SARS CoV-2, MERS and SARS Coronavirus, their protein sequences were submitted to the Motif Scan server (https://myhits.sib.swiss/cgi-bin/motif_scan). A unique *Staphylococcus* coagulase repeat

Abbreviations: SARS, CoV-2: Severe Acute Respiratory Syndrome Corona Virus-2; SARS, Severe Acute Respiratory Syndrome; MERS, Middle East Respiratory Syndrome; Efb, Extracellular fibrinogen binding; vWBP, von Willebrand factor Binding Protein; MASP-2, MBL-associated serine protease-2

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motif was identified in the N-protein of SARS CoV-2. Coagulase motifs are present as multiple repeats in the C-terminal region of the coagulase protein (GenBank: AKJ16095.1) of *Staphylococcus aureus* (*S. aureus*), which is known to interact with fibrinogen of the host. This motif is also present in an extracellular protein Efb (Extracellular fibrinogen binding) (GenBank ADJ67155.1) of *S. aureus*. Peptides representing these motifs bind to fibrinogen with moderate to high affinity [5]. Sequence alignment tool EMBOSS Needle (https://www.ebi.ac.uk/Tools/psa/emboss_needle/) was used to check the degree of homology between coagulase motifs found in SARS CoV-2 N-protein and that of *S. aureus* proteins. There is significant homology (Identity: 33.3% and Similarity: 40.7%) between the motifs found in peptides Coa-RV1 (Coagulase, 610–636 aa) and Efb (68–94aa). The coagulase motif found in SARS CoV-2 N-protein (256–282 aa, **Motif I**) had higher degree of homology to Coa-RV1 (Identity: 45.2% and Similarity: 48.4%) than that of Efb. When aligned using BLAST (Basic Local Alignment Search Tool), another region of the N-protein (375–409 aa, **Motif II**) was homologous to the sequence present in the D2 domain (209–241aa) of vWBP (von Willebrand factor Binding Protein) (GenBank: BBJ17761.1) of *S. aureus* (Identity: 29.7% and Similarity: 62.2%). D1 (1–131 aa) and D2 (132–244 aa) domains of vWBP interact with prothrombin and fibrinogen. Both coagulase and vWBP are well known for their role in formation of blood clots in the host during an *S. aureus* infection [6]. Therefore, N-protein can potentially interact with the host fibrinogen and prothrombin like these proteins of *S. aureus*. Motifs I and II on N-protein could regulate the functions of fibrinogen and perhaps prothrombin (via Motif II) to activate the coagulation cascade and alter signalling pathways to escape from the host immune response.

Evaluation of the hypothesis

Fibrinogen interaction with coagulase and Efb of *S. aureus* is well established [5,7]. The C-terminus of coagulase have five motifs (repeats) that can interact with fibrinogen (Coa-RI to Coa-RV). A homologous motif is also reported in Efb. Detailed studies involving peptides that represent motifs of coagulase and Efb have confirmed that their affinity of fibrinogen is relatively high. Of all the repeats in coagulase, Coa-RI (506–532 aa) peptide bound to fibrinogen with maximum affinity ($K_D \sim 77$ nM), and peptides CoaRI3 (502–528 aa, $K_D \sim 140$ nM) as well as Coa-RV1 (610–636 aa, $K_D \sim 167$ nM) bound with relatively moderate affinity [5]. The nomenclature of the peptides is as mentioned by Ko et al. [5]. The fibrinogen interaction motif of Efb is also homologous to Coa-RV1. As mentioned earlier the Motif I of N-protein is homologous to Coa-RV1, where the degree of homology is much higher than that of Efb and Coa-RV1. With these observations, it is very much likely that N-protein of SARS CoV-2 can interact with fibrinogen through its Motif I.

Motif II of N-protein was found to be homologous to a part of D2 domain of vWBP. D2 domain is known for its interaction with both fibrinogen and prothrombin [6]. The exact sequence/motif is yet to be determined that is involved interaction. Considering the partial homology of Motif II with D2 domain, N-protein may be capable of associating with prothrombin also. From these observations, there is a large probability of N-protein may regulate similar functions as the secretory proteins of *S. aureus* in the host.

Possible implications of N-protein-fibrinogen interaction

Many SARS CoV-2 infected patients have blood clots and coagulation disorders in advanced stages which have proved to be fatal [1]. It is well known that coagulase and vWBP of *S. aureus* can activate prothrombin non-proteolytically leading to conversion of associated fibrinogen to fibrin to generate blood clots and abscesses. Formation of a fibrin layer protects the pathogen from opsonophagocytic clearance of the immune system [8]. Additionally, interaction of fibrinogen with $\alpha_M\beta_2$ /Mac-1 integrin is crucial for host inflammatory response, which

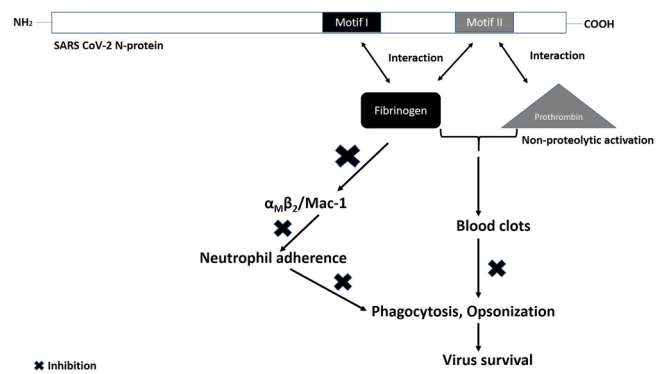


Fig. 1. Implications of the N-protein interaction with fibrinogen and prothrombin. A schematic showing the possible consequences due to interaction of N-protein with fibrinogen and prothrombin.

helps in clearance of the pathogen [9,10]. Efb-fibrinogen interaction inactivates $\alpha_M\beta_2$ /Mac-1 integrin. Two modes of inactivation have been proposed- (i) A conformational change in $\alpha_M\beta_2$ /Mac-1 integrin due to the binding of fibrinogen, resulting in inactivation. (ii) A steric hindrance that prevents interaction of fibrinogen due to its engagement with Efb [7]. This prevents the adherence of neutrophils and prevents the phagocytosis of the pathogen [7]. The C-terminus of Efb interacts with C3 protein to activate complement pathway during infection. Efb creates a fibrinogen shield preventing opsonins like C3b and Fc-region of IgG, inhibiting phagocytosis by neutrophils [11]. N-protein of SARS CoV-2 activates the complement system through serine protease MBL-associated serine protease-2 (MASP-2) [12]. Considering the homology to Efb, it is also possible that the fibrinogen-binding motif of N-protein may inhibit cell adherence of neutrophils, prevent Fc or C3b mediated opsonization and phagocytosis (Fig. 1).

Conclusion

The role of the fibrinogen-binding motif of N-protein in formation of blood clots and mimicking functions of Efb for pathogen survival in host will be investigated. The affinity of the motif towards fibrinogen will play a major role in regulating the functions of fibrinogen and in turn the host immune system. At this juncture, the knowledge of existence of such a motif will help investigators to further probe and establish its role in host-pathogen interaction and provide insights for immunomodulation-based therapies for SARS CoV-2 infections.

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