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STRUCTURE, PATHWAY PREDICTION FOR FUNCTIONAL ELEMENTS OF E2 GLYCOPROTEIN PRECURSOR IN SARS-COV-2 TO ANALYZE IT'S ROLE IN COVID-19 DISEASE

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ABSTRACT

The COVID-19 is caused by SARS-CoV-2 (Severe Acute Respiratory Syndrome Corona Virus -2). It has became a major global health issue of 2019-20.[2] It's single stranded RNA virus of approximately 30 kb genomic length. Due to positive sense it has ability of rapidly translate it's genome in the host cell.[9] Reason for spread of disease from human to human is via droplets or direct contact with infected person. The World Health Organization (WHO) declared COVID-19 outbreak as sixth public health emergency of international concern (PHEIC) on 30 January 2020.[5] More research is needed to identify the structural as well as functional characteristics of SARS-CoV-2's proteins that are essential for pathogenic mechanism.[2] In this study our aim is to analyze the structural features of E2 glycoprotein precursor which is trans membrane protein of SARS-CoV-2 by performing structure validation. Further it has been observed het group NAG (N-Acetyl-D-Glucosamine) in the protein's structure by predicting secondary structure features, which has the ability to induce the cell apoptosis mechanism by it's catalytic activity.[14] The said study is to implement the correlation between activity of NAG group of the protein inside the cellular environment and their possible adverse effects on human body. This will help us in identification of the possible functional activity of E2 glycoprotein precursor in pathogenesis with the help of advance computational biology named Bioinformatics. Swiss model (Automated protein structure homology modeling server), PDBsum, KEGG Database above said server and program are used to analyze the activity.[1,10,11,12,13]

KEYWORDS: COVID-19, E2 Glycoprotein precursor, NAG, Hydrogen peroxide, Apoptosis, Structure Prediction, KEGG Pathway.

INTRODUCTION

1. 2019-n-CoV or SARS-CoV-2 or COVID-19

Corona viruses (CoVs) are the largest group of viruses belongs to the Nidovirales order. Which is further divided into four families namely Coronaviridae, Arteriviridae, Mesoniviridae, and Roniviridae. In which Coronaviridae family is further subdivided into four genera are *alpha*, *beta*, *gamma*, and *delta* corona viruses.[4] There are currently seven corona virus species are known to cause disease in humans out of that four (229E, OC43, NL63 and HKU1) only cause common cold which is a mild symptom.[9] Remaining three viruses are SARS-CoV (emerged in 2002-03), MERS-CoV (The Middle East respiratory syndrome emerged in 2012) and SARS-CoV-2 (Severe Acute Respiratory Syndrome Corona virus-2) which can cause severe illness. World Health Organization announced new name for the epidemic disease caused by SARS-CoV-2 as 'COVID-19' and declared it as the sixth public health emergency of international concern. SARS-CoV-2 is positive sense (doesn't require transcription for making m-RNA copies), single stranded enveloped RNA virus belonging to genus *Betacorona virus*.[5] It's approximately 30 kb in genomic length and known to be a largest RNA virus.[4] Origin of this virus is still not properly understood but, genomic analysis suggests that it is probably evolved from a strain found in bats with 82-89% nucleotide identity.[9] Common symptoms are fever and cough. Typical symptom of this disease is pneumonia which is classified into three major stages like mild, severe and critical disease. In which mild disease have non-pneumonia and mild pneumonia symptoms occurs in 81% of the cases. Severe disease include dyspnea with respiratory frequency of (>=30) breaths per min occurs in 14% of the patients. Critical disease shows respiratory failure, multiple organ dysfunction (MOD) or failure (MOF) occurs in 5% of patients.[5] More research is needed to understand the structural as well as functional characteristics of SARS-

CoV-2's proteins that are involved in pathogenic mechanism.

2. E2 Glycoprotein precursor

Corona virus contains four main structural proteins as spike(S), membrane (M), envelope (E) and nucleocapsid (N) proteins [Figure no 01]. E2 Glycoprotein precursor is 1255 amino acid long polypeptide chain protein encoded by 'S' gene.[4] Topology is not completely understood but most of the data suggests that it is a trans-membrane protein. E proteins facilitates assembly and release of virus but they also has other unrevealed functions too.[4] We have study E2 glycoprotein precursor implementing bioinformatics methods to analyze it's role in causing pathogenesis.

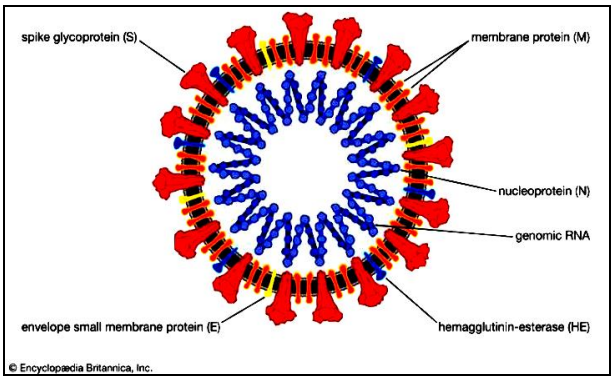
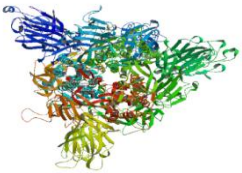


Figure 01: Figure showing diagrammatic representation for structural proteins of SARS-CoV-2.

3. Structure prediction

Protein secondary and tertiary structure prediction provides basis of understanding it's function.[11] There are three methods available for prediction of tertiary structure of protein are Homology modeling, AB initio and threading. We have predicted tertiary structure of E2 glycoprotein precursor by using homology modeling method. Homology modeling builds atomic model from amino acid sequence of protein based on sequence homology with known experimentally determined structures available in the database like PDB (Protein Data Bank).[12] The structure of E2 glycoprotein precursor is predicted by using 'SWISS model' which is online tool provided by Expasy server (Expert Protein Analysis System). Three dimensional structure of E2 glycoprotein precursor is predicted. Q-mean (Qualitative Model Energy ANalysis) Z-score provides 'degree of nativeness' of the structural features observed in the model on global scale.[10] Q-mean z-score near to zero (-2.21) indicate good agreement between the model structure and experimental structure of similar size [Table no 01]. Protein's secondary structure features are also predicted by using PDBsum web server by EMBL-EBI.[1] PDBsum is a web server which provides structural information on the entries in the Protein Data Bank (PDB). It shows the molecules that make the structure like chains, ligands, ion metals etc.[12] We found that there are three chains A, B, C which are connected to each other by the various bonds of interactions to form this complex macromolecule E2 glycoprotein precursor [Figure no 02]. We also found a het group 'NAG' in the predicted structure of E2 glycoprotein precursor [Figure no 03,04]. Further study has been done on the catalytic activity of this het group NAG in order to predict it's functional involvement in the pathogenesis.

Table 01: Table showing Accession ID, Predicted structure and Q-mean Score of E2 glycoprotein precursor.

Sr No.	Name	Accession ID	Predicted structure	Q-mean score
1	E2 glycoprotein precursor [Severe acute respiratory syndrome-related coronavirus]	NP_828851.1		-2.21

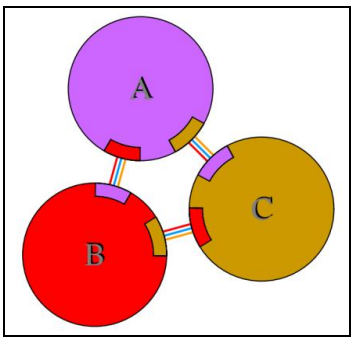


Figure 02: Figure showing interaction between three chains A, B and C of E2 glycoprotein precursor [Red line= salt bridges, Blue line= hydrogen bonds, Orange line= non-bonded interaction]

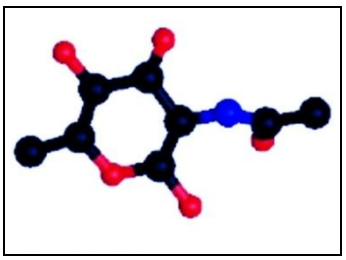


Figure 03: Figure showing three dimensional structure of het group NAG found in E2 glycoprotein precursor.

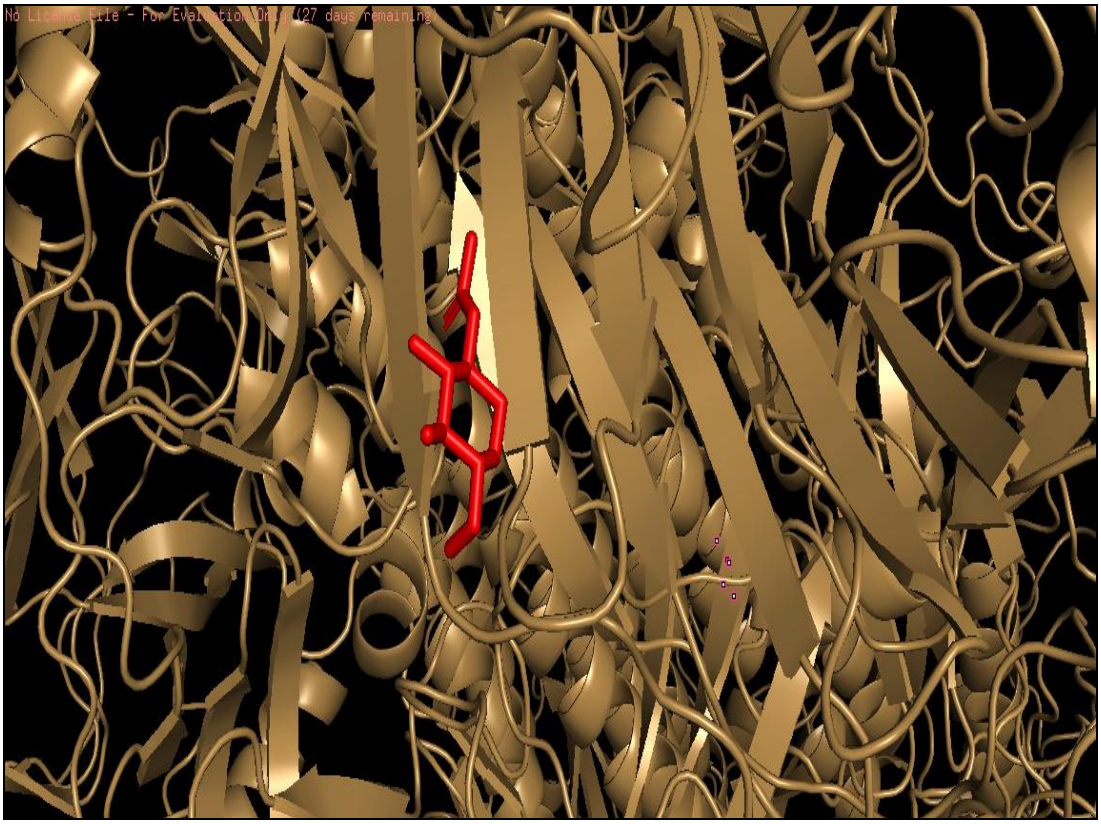


Figure 04: Figure showing het group NAG found in predicted structure of E2 glycoprotein precursor visualized in 'PyMol' structure visualization software (NAG group shown in Red color).

4. NAG group and it's reaction with O_2 and H_2O

NAG group have different names such as N-Acetyl-D-Glucosamine, N-Acetylchitosamine, 2-Acetamido-2-deoxy-D-glucose and GIcNAc. It's a D-saccharide having molecular formula $C_8H_{15}NO_6$. Then we study it's reaction with oxygen (O_2) and water (H_2O) which forms N-Acetyl-D-Glucosaminate and Hydrogen peroxide (H_2O_2) [Figure no 05].

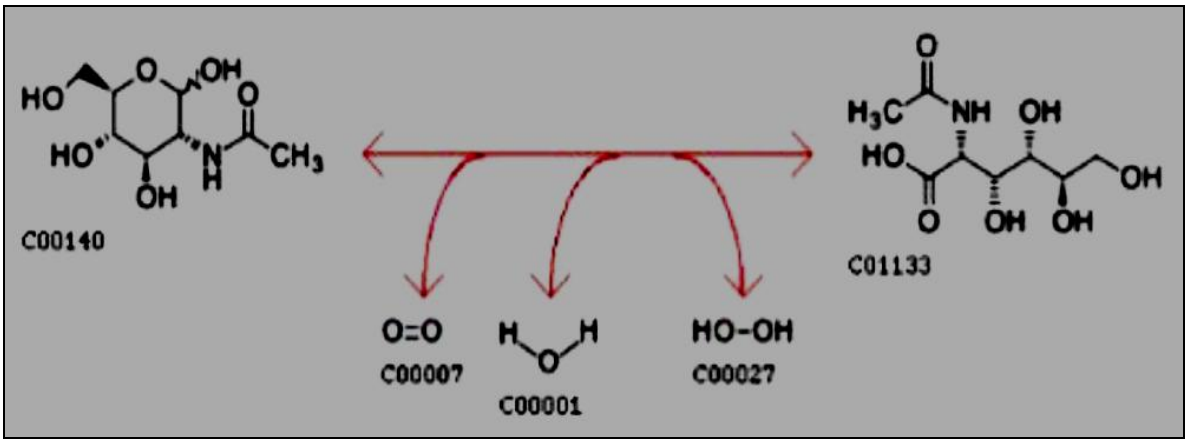


Figure 05: Figure showing reaction of NAG Group with oxygen and water to form Hydrogen peroxide.

5. H_2O_2 and it's role in apoptosis

In above reaction hydrogen peroxide is liberated as a by product. Apoptosis is a chemical induced cell death.[3] Hydrogen peroxide is cell damaging agent produced by the cell itself in normal metabolism.[6] Hydrogen peroxide induces cell apoptosis by triggering the autophagy cell death called as lysosomal degradation process or by mitochondrial pathway.[8] Hydrogen peroxide causes toxicity by three main mechanisms as corrosive damage, oxygen gas formation, and lipid peroxidation.[7] It's high concentration may result in local tissue damage and may lead to organ damage and failure. Excessive formation of hydrogen peroxide may cause oxidative stress and disease too. The pathway for hydrogen peroxide's role in cell apoptosis mechanism has been predicted with the help of KEGG Database.[13] [Figure no 06].

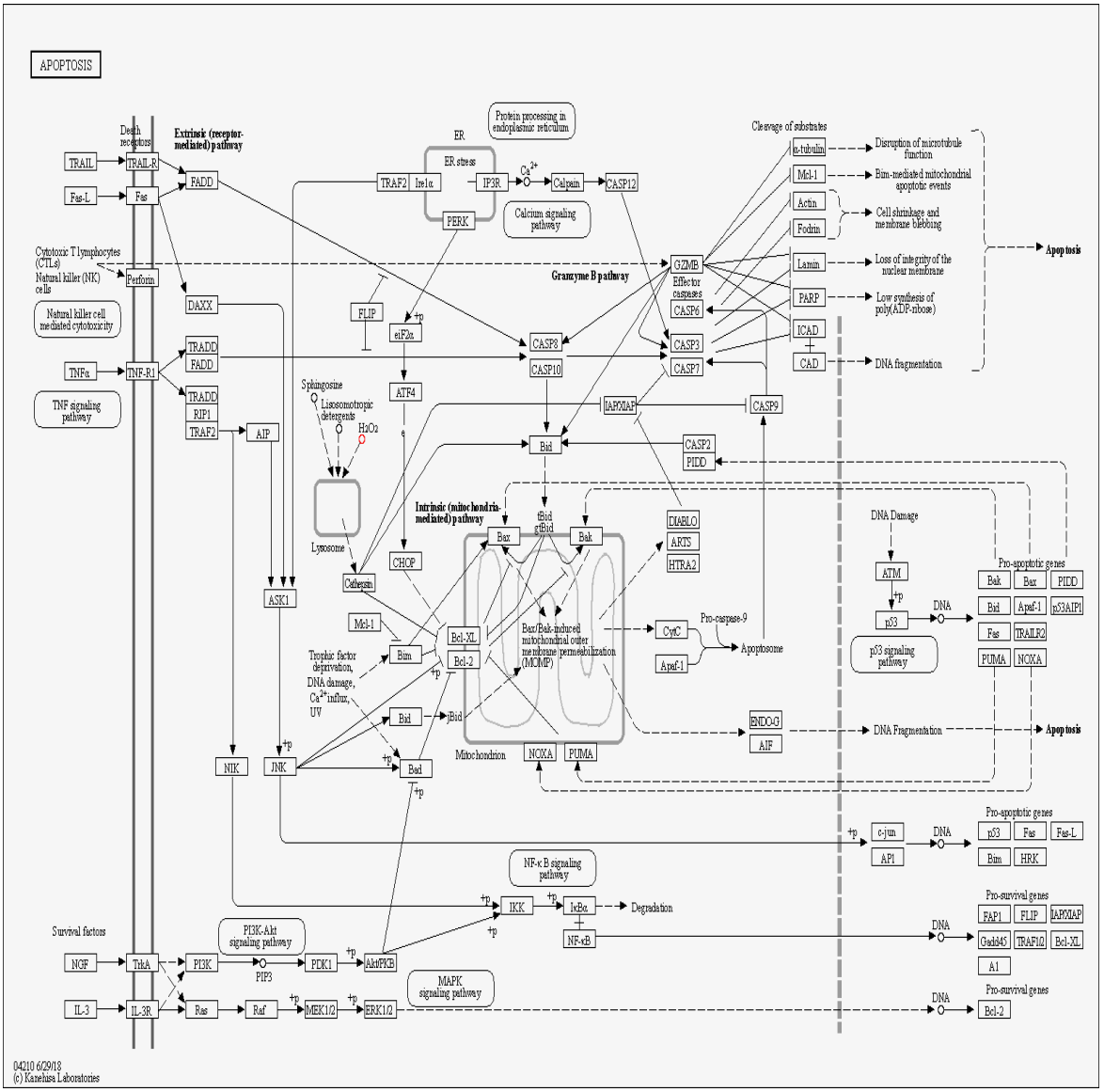


Figure 06: Figure showing predicted pathway of hydrogen peroxide inside the cytoplasm of human cell from KEGG database (hydrogen peroxide is highlighted in Red color).

CONCLUSION

This study is based on secondary and tertiary structure prediction of E2 glycoprotein precursor which is trans-membrane protein of SARS-CoV-2. Q-mean score which is '-2.21' shows good quality of predicted structure. Further secondary structure features prediction helped to pull out the functional elements in the protein. We found het group NAG in the predicted structure which can react with oxygen and water molecules to form oxidative agent hydrogen peroxide in the cell. Hydrogen peroxide induces the mechanism of apoptosis by triggering the lysosomal degradation process or by mitochondrial pathway. Hydrogen peroxide formed by this protein by the activity of it's het group NAG may produce it in excess amount because, it's not produced by the host's metabolism itself (produced by SARS-CoV-2's trans membrane protein i.e. E2 glycoprotein precursor). If concentration of hydrogen peroxide increases inside the infected cell of the body it may result in oxidative stress and cause of disease. From all results we are reached to conclude that E2 glycoprotein precursor may cause an adverse effect by producing hydrogen peroxide. We can say that inducing apoptosis in normal healthy cells can be the functional activity of E2 glycoprotein precursor. This activity of the protein can be one the reason for causing pneumonia by damaging normal cells of the lungs. By inhibiting its activity may help in prevent the abnormal apoptosis in healthy cells and can save the patient from causing pneumonia and ultimately COVID-19.

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