Observation of structural dynamics with change in pH of COVID-19 spike protein RBD domain

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**Introduction:**

A novel coronavirus in December 2019, contributed to a series of acute atypical respiratory diseases in Wuhan, Hubei Province, China. The virus is predominantly spread from human to human via contact and respiratory droplets and is highly infectious. This outbreak was later classified as a global pandemic by WHO and is already responsible for over half a million deaths worldwide. Epidemiological studies and various guidelines have depicted that elderly people, pregnant females, and children are more susceptible to the disease. In the univariable analysis, the presence of coronary artery disease, diabetes, and hypertension have also been considered to be hazardous. A majority of patients died from multi-organ failure, respiratory failure, shock, and acute respiratory distress syndrome (ARDS). Other less common reasons for death are myocardial injury, heart failure, coagulation dysfunction, and acute kidney injury. Angiotensin-converting enzyme 2 (ACE2) is the cellular receptor for SARS-CoV and SARS-CoV-2. ACE2 is primarily expressed in the upper respiratory system, alveolar epithelial cells of the lungs, heart, endothelial cells, kidney tubular epithelium, enterocytes, and the pancreas.

Proximal serine proteases help in the entry of cellular viruses into the cell through the endosomal pathway via the release of spike fusion proteins. The protease is involved in priming and then cleavage of the spike protein after binding to ACE2. For SARS-CoV-2, low pH in the presence of proteases such as cathepsin-L favors its genome into the cytosol where viral replication takes place. Hydrogen ions are the most reactive ions present in living organisms. All biological solutions have a certain hydrogen ion concentration

arising from the balance between deprotonation and protonation reactions of water, weak acids, and weak bases.

Cell survival is conditional on the maintenance of a favorable acid-base balance (pH). Due to intensive respiratory CO2 and lactic acid production, cancer cells are manifested continuously to large acid-base fluxes, that disturbs pH. In general, tumors are more acidic than normal tissues with median pH values of about 7.0 in tumors and 7.5 in normal tissues. Acute respiratory diseases such as acute hypercapnic respiratory failure are associated with lowering of pH over the short timeline of its development, but chronic respiratory failure which develops over a much longer duration allows sufficient time for renal compensation and an increase in bicarbonate concentration thus bringing the pH towards neutrality. In patients having diabetic ketoacidosis, a condition of life-threatening acute metabolic complication of uncontrolled diabetes (type-1), pH ≤ 6.9 is observed.

The present investigation was designed keeping in view all the pathological conditions and the role of pH in the replication of the virus.

The **physiological pH** of a few organs/tissues are given below:

|  |  |  |
| --- | --- | --- |
| Tissue | pH | Source |
| Lung | 7.38-7.42 | https://microbewiki.kenyon.edu/index.php/Lungs |
| Kidney | 7.38-7.42 |  |
| Small Intestine | 7.2-7.5 | [https://www.news-medical.net/health/pH-in-the-Human-Body.aspx#](https://www.news-medical.net/health/pH-in-the-Human-Body.aspx) |
| Colon | 7.0-7.5 | Bawa, Priya, et al. "Stimuli-responsive polymers and their applications in drug delivery." *Biomedical materials* 4.2 (2009): 022001. |
| Duodenum | 4.8-8.2 | Bawa, Priya, et al. "Stimuli-responsive polymers and their applications in drug delivery." *Biomedical materials* 4.2 (2009): 022001. |
| Pancreas | 8.0-8.3 | Melamed, Peter, and Felix Melamed. "Chronic metabolic acidosis destroys pancreas." *JOP. Journal of the Pancreas* 15.6 (2014): 552-560. |
| Rectum | ~7.9 | Bitterman, Wilhelm, et al. "Contact pH of rectal mucosa in humans and dogs." *Diseases of the Colon & Rectum* 12.2 (1969): 96-98. |
| Liver (Bile) | ~7.8 | Melamed, Peter, and Felix Melamed. "Chronic metabolic acidosis destroys pancreas." *JOP. Journal of the Pancreas* 15.6 (2014): 552-560. |
| Testis (luminal pH) | 6.6-7.8 | Shum, Winnie WC, et al. "Regulation of luminal acidification in the male reproductive tract via cell–cell crosstalk." *Journal of Experimental Biology* 212.11 (2009): 1753-1761. |
| Fallopian tube | ~7.94 | Ng, Ka Ying Bonnie, et al. "In vivo oxygen, temperature and pH dynamics in the female reproductive tract and their importance in human conception: a systematic review." *Human reproduction update* 24.1 (2018): 15-34. |

**Experimental Setup:**

The electron microscopically derived structure coordinates of Angiotensin-converting enzyme 2 (ACE2) and receptor binding domain (RBD) of the Spike Glycoprotein (SG) were taken from the 6LZG.pdb **(Protein Data Bank)**. Protein preparation wizard of Schrodinger Maestro was used for various corrections in the chosen experimental structure including the missing bond orders, missing side chains, addition of Hydrogen bonds, conversion of selenomethionine into methionine *etc.* The resulting structure was corrected for unusually close contacts. The protonation state of the amino acid side chains of resulting protein was optimised as per pH 6.6, 7.0 and 7.4 using the **PROPKA’s algorithm** using PROPKA tool. Three simulation conditions were considered for comparative molecular dynamics at different pH *i.e.* monomeric ACE2-RBD complex, dimeric ACE2-RBD complex, monomeric ACE2 Apo. The resulting structures were minimised and then solvated using the TIP3P water model with periodic boundary conditions of 10 angstrom and orthorhombic box geometry. The overall system was neutralised using an appropriate number of counters Na+ and Cl- as calculated by the system builder module of Desmond Suite. The difference in counter ions used to neutralise the system was recorded to calculate the number of amino acids with altered protonation state(s). Physiological salt concentration of 0.15 mM was maintained by the addition of an appropriate number of Na+ and Cl- ions. VMD tool was used to visualize these amino acids with altered protonation state(s) in the mentioned pH conditions. Solvated systems thus generated were subjected to preparatory molecular dynamics simulations (MDS) using default parameters. All production MDS were conducted at 310 K and ~1 Bar for 100 ns, the trajectories and energy were recorded every 1.2 ps and 10 ps, respectively. A total of 10,000 conformations (*i.e.* trajectory frames) were generated for each MDS.

*Eq3.1*

*or*

*Eq3.2*

*Eq. 3.3*

**Results:**

Following MDS, the trajectory was analysed using VMD to determine possible states of active and inactive conformation, interactions and amino acid hotspots at pH 6.6 and 7.4. The results of the simulation are summarized in the tables below:

1. At pH 6.6:
2. **Interactions and Total Stabilizing Energy for pH 6.6**

|  |  |
| --- | --- |
| **Hydrogen Bond** | **0.00 kJ/mol** |
| **Energy (Electrostatic Energy)** | **-2.17 kJ/mol** |
| **Van der Waals Energy** | **-145.28 kJ/mol** |
| **Total Stabilizing Energy** | **-147.45 kJ/mol** |
| **Number of interface residues** | **76** |
| **Normalized Energy per residue** | **-1.94 kJ/mol** |
| **No. of Short Contacts** | **0** |
| **No. of Hydrophobic Interactions** | **3** |
| **No. of van der Waals Pairs** | **1917** |
| **No. of Salt Bridges** | **6** |
| **No. of Potential Favourable Electrostatic Interactions** | **3** |
| **No. of Potential Unfavourable Electrostatic Interactions** | **4** |

1. **Amino Acid Residues at interface :**

|  |  |
| --- | --- |
| **ChainB (ACE2)** | **ChainE (RBD)** |
| B101GLN B20ACE B21ILE B22GLU B23GLU B24GLN B25ALA B26LYS B27THR B28PHE B29LEU B30ASP B31LYS B32PHE B34HIS B35GLU B38ASP B39LEU B42GLN B64ASN B67ASP B68LYS B71ALA B72PHE B74LYS B75GLU B76GLN B79LEU B81GLN B82MET B83TYR B84PRO B85LEU B86GLN B87GLU B89GLN | E403ARG E405ASP E417LYS E418ILE E446GLY E449TYR E453TYR E454ARG E455LEU E456PHE E457ARG E458LYS E473TYR E474GLN E475ALA E476GLY E477SER E478THR E479PRO E483VAL E484GLU E485GLY E486PHE E487ASN E488CYS E489TYR E490PHE E E491PRO E492LEU E493GLN E494SER E495TYR E496GLY E498GLN E499PRO E500THR E501ASN E502GLY E504GLY E505TYR |

1. **Potential Hotspots**

**Res Num Res Name Chain**

21 ILE B

489 TYR E

24 GLN B

455 LEU E

83 TYR B

487 ASN E

486 PHE E

84 PRO B

493 GLN E

1. **INTERACTION WISE RESIDUE CLASSIFICATION FOR pH 6.6**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Potential Hydrophobic Interactions** | | | | | | | | |
| **RESIDUE-1** | | | | **RESIDUE-2** | | | | **Distance (D-A)  Å** |
| **Res Num** | **Res Name** | **ACE2**  **(Chain B)** | **Atom Name** | **Res Num** | **Res Name** | **RBD**  **(Chain E)** | **Atom Name** |
| 21 | ILE | B | CB | 479 | ALA | E | CB | 3.52 |
| 21 | ILE | B | CB | 485 | TYR | E | CB | 5.66 |
| 28 | PHE | B | CB | 455 | LEU | E | CB | 6.05 |
| **Potential Favorable Electrostatic Interactions** | | | | | | | | |
| **RESIDUE-1** | | | | **RESIDUE-2** | | | | **Distance  Å** |
| **Res Num** | **Res Name** | **ACE2**  **(Chain B)** | **Atom Name** | **Res Num** | **Res Name** | **RBD**  **(Chain E)** | **Atom Name** |
| 23 | GLU | B | CB | 458 | LYS | E | CB | 7.82 |
| 30 | ASP | B | CB | 417 | LYS | E | CB | 9.05 |
| 35 | GLU | B | CB | 417 | LYS | E | CB | 9.76 |
| **Potential Unfavorable Electrostatic Interactions** | | | | | | | | |
| **RESIDUE-1** | | | | **RESIDUE-2** | | | | **Distance  Å** |
| **Res Num** | **Res Name** | **ACE2**  **(Chain B)** | **Atom Name** | **Res Num** | **Res Name** | **RBD**  **(Chain E)** | **Atom Name** |
| 31 | GLU | B | CB | 417 | LYS | E | CB | 8.83 |
| 34 | HIS | B | CB | 417 | LYS | E | CB | 7.91 |
| 35 | GLU | B | CB | 405 | ASP | E | CB | 9.67 |
| 38 | ASP | B | CB | 405 | ASP | E | CB | 9.70 |
| **Potential Salt Bridges** | | | | | | | | |
| **RESIDUE-1** | | | | **RESIDUE-2** | | | | **Distance  Å** |
| **Res Num** | **Res NameTLR5** | **ACE2**  **(Chain B)** | **Atom Name** | **Res Num** | **Res Name** | **RBD**  **(Chain E)** | **Atom Name** |
| 23 | GLU | B | OE1 | 458 | LYS | E | NZ | 3.92 |
| 23 | GLU | B | OE2 | 458 | LYS | E | NZ | 2.64 |
| 35 | GLU | B | OE1 | 403 | ARG | E | NH1 | 2.67 |
| 35 | GLU | B | OE1 | 403 | ARG | E | NH2 | 3.31 |
| 35 | GLU | B | OE2 | 403 | ARG | E | NH1 | 3.63 |
| 35 | GLU | B | OE2 | 403 | ARG | E | NH2 | 2.82 |

1. At pH 7.4:
2. **Interactions and Total Stabilizing Energy for pH 7.4**

|  |  |
| --- | --- |
| **Hydrogen Bond** | **0.00 kJ/mol** |
| **EnergyElectrostatic Energy** | **1.52 kJ/mol** |
| **Van der Waals Energy** | **-174.65 kJ/mol** |
| **Total Stabilizing Energy** | **-173.12 kJ/mol** |
| **Number of interface residues** | **92** |
| **Normalized Energy per residue** | **-1.88 kJ/mol** |
| **No. of Short Contacts** | **0** |
| **No. of Hydrophobic Interactions** | **2** |
| **No. of van der Waals Pairs** | **2490** |
| **No. of Salt Bridges** | **1** |
| **No. of Potential Favourable Electrostatic Interactions** | **2** |
| **No. of Potential Unfavourable Electrostatic Interactions** | **3** |

1. **Amino Acid Residues at interface :**

|  |  |
| --- | --- |
| **ChainB (ACE2)** | **ChainE (RBD)** |
| B20ACE B21ILE B23GLU B24GLN B25ALA B26LYS B27THR B28PHE B29LEU B30ASP B31LYS B322ASN B323MET B324THR B325GLN B327PHE B32PHE B330ASN B33ASN B34HIS B351LEU B352GLY B353LYS B354GLY B355ASP B356PHE B357ARG B35GLU B36ALA B37GLU B386ALA B387ALA B388GLN B389PRO B38ASP B390PHE B393ARG B39LEU B41TYR B42GLN B45LEU B72PHE B75GLU B76GLN B78THR B79LEU B80ALA B81GLN B82MET B83TYR B84PRO | E403ARG E405ASP E408ARG E421TYR E446GLY E448ASN E449TYR E453TYR E455LEU E456PHE E457ARG E458LYS E473TYR E475ALA E476GLY E477SER E478THR E483VAL E484GLU E485GLY E486PHE E487ASN E488CYS E489TYR E490PHE E491PRO E492LEU E493GLN E494SER E495TYR E496GLY E497PHE E498GLN E499PRO E500THR E501ASN E502GLY E503VAL E504GLY E505TYR E506GLN |

1. **Potential Hotspots**

**Res Num Res Name Chain**

353 LYS B

505 TYR E

486 PHE E

500 THR E

31 LYS B

501 ASN E

489 TYR E

502 GLY E

493 GLN E