The influence of mutations in the SARS-CoV-2 RBD on the strength of binding to the ACE2 receptor and on the spread of the virus in humans

Danuta Witkowska[1], Mariusz Kubus[2]

[1]Institute of Health Sciences, University of Opole, Opole, Poland

[2]Centre of Education and Mathematics Applications, Opole University of Technology, Opole, Poland

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Introduction

Studying variants of the SARS-CoV-2 virus remains crucial for several reasons. First, viral variants can exhibit changes in transmissibility, potentially leading to increased spread within populations. Second, certain variants have the potential to evade immunity from previous infections or vaccinations. Analyzing specific mutations contributes to our understanding of viral evolution, offering insights into how viruses adapt to their environments. Mutations in the receptor-binding motif of the SARS-CoV-2 spike protein, particularly N501Y and E484K, have garnered significant attention due to their potential impact on ACE2 binding kinetics and strength. While there is substantial evidence supporting the notion of increased viral transmission associated with N501Y and partial immune evasion associated with E484K, reaching definitive conclusions can be complicated by the diversity of variants and the different techniques used by researchers. By employing statistical methods, we could effectively determined whether the experimental results significantly differed from the theoretical predictions regarding the impact of the above-mentioned mutations on the binding affinity to the ACE2 receptor protein.

Then, using isothermal titration calorimetry (ITC), we investigated the impact of the single or combined mutations (N501Y and E484K) on the binding of the viral receptor-binding motif (RBM) fragments to the human ACE2 receptor, particularly focusing on the affinity and enthalpy of this interaction.

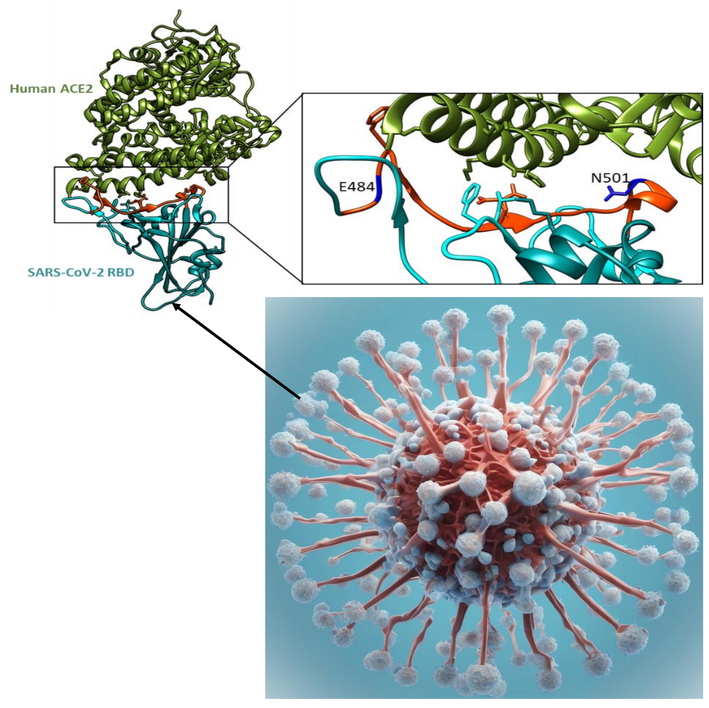


Fig.1. Overall structure of SARS-CoV-2 RBD bound to ACE2 receptor. Analysed fragment of RBM is shown in red, the variable amino acids are in blue. Visualised by USCF CHIMERA. PDB: 6M0J.

Methods

All calculations were performed in R program. The Silhouette Index was calculated for evaluating different clustering algorithms and determining the optimal number of clusters. Isothermal titration calorimetry measurements were performed at 25 °C and a pH of 7.4 on a MicroCal PEAQ Isothermal Titration Calorimeter (Malvern Panalytical Ltd., Malvern, UK). After the device was stabilised at 25 °C, 40 µL of buffered peptide solutions were used to titrate 200 µL of buffered ACE2 solutions (the concentration was initially about 10 times lower than that of RBD) by 19 consecutive injections with an interval of 150 s between each drop and a stirring speed of 750 rpm (each test was repeated a few times). The reference cell was filled with deionised water. Data were fitted using MicroCal PEAQ-ITC analysis software.

Results

The experimental results obtained in the articles A,B,C,D,E are expressed in K_d measure, whereas theoretical results from F,G,H,I in ΔG measure. In both cases, we took relative differences between these measures and WT, so that to obtain comparability of these results:

$$RD = \frac{x - WT}{|WT|} \cdot 100\%$$

where *x* is dissociation constant for experimental results or binding energy for theoretical results. In this way, we have a measure, which is expressed in %. Because both measures K_d and ΔG are destimulants, the minus sign of *RD* shows that the strength of binding has grown (Fig.2 and Fig.3).

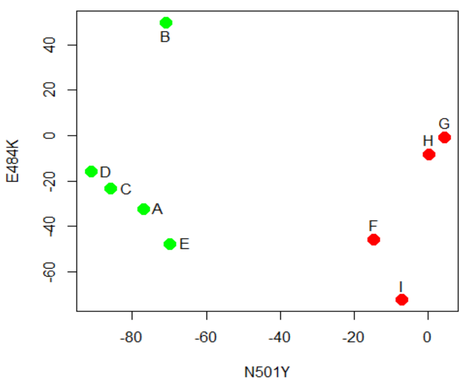


Fig. 2. Scatterplot of the articles in normalized plane spanned by *RD* measure for N501Y and E484K. The results of experimental researches in green, and theoretical results in red.

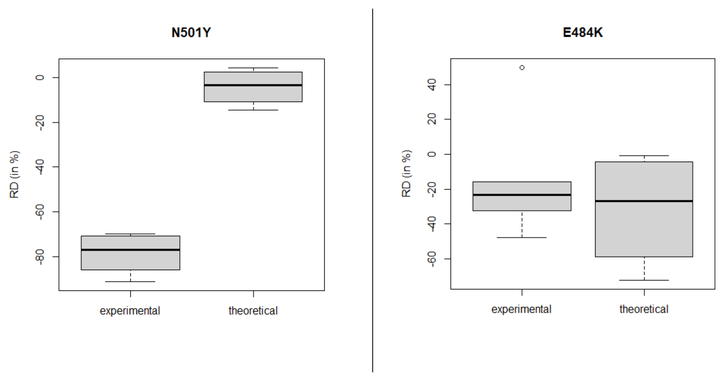
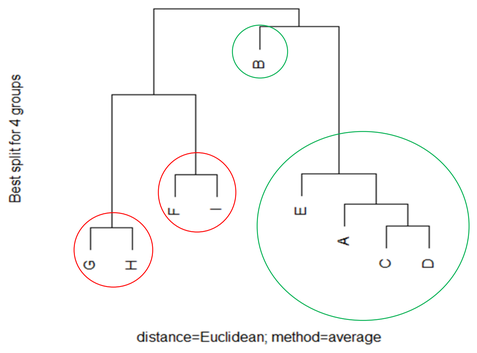


Fig. 3. The comparison of RD (relative differences) for experimental and theoretical results.

484 501

Ac-GVEGFNCYFPLQSYGFQPTNG-NH$_2$ Wuhan (WT)
Ac-GVEGFNCYFPLQSYGFQPTYG-NH$_2$ Alpha
Ac-GVKGFNCYFPLQSYGFQPTYG-NH$_2$ Beta/Gamma
Ac-GVKGFNCYFPLQSYGFQPTNG-NH$_2$ Zeta



CLUSTER ANALYSIS

Cluster analysis is a method of finding groups in data. The objects (here articles) in one group should be similar to each other, while objects from different groups should be significantly different. Similarity is defined with respect to some features (here RD measures in N501Y and E484K), and its measure is mathematical distance (Fig.4).

Fig. 4. The similarities of the article' results are considered in 2-dimensional space of RD measure for N501Y and E484K. According to the silhouette index, the optimal number of groups is four.

Table 1. Silhouette index for a particular splits (k – number of groups). Its maximum points out the optimal number of groups, and the value 0.60141 indicates quite strong group structure.

k = 2	k=3	**k=4**	k=5	k=6
0.49679	0.52270	**0.60141**	0.43204	0.31535

Final groups:
A B C D E F G H I
1 2 1 1 1 3 4 4 3

ITC results

Table 2. Differences in the hACE2 receptor binding thermodynamics between studied RBM fragments of five SARS-CoV-2 variants, at pH 7.4 and 25 °C.

RBM fragments	K_{dITC} [µM]	N	ΔH_{ITC} [kcal/mol]	$-T\Delta S_{ITC}$ [kcal/mol]
WT	4.74 ± 1.45	0.96 ± 0.13	– 3.4 ± 0.68	– 3.94
Alpha	2.15 ± 0.83	0.96 ± 0.14	– 3.29 ± 0.70	– 4.44
Beta/Gamma	0.31 ± 0.08	0.91 ± 0.02	– 2.28 ± 0.09	– 6.61
Zeta	0.81 ± 0.06	0.76 ± 0.03	– 4.26 ± 0.24	– 4.06

Conclusions

Our calculations indicate that the results reported in the literature (References A-I), which examine the impact of the E484K and N501Y mutations on binding affinity, vary based on the methodological approach employed, whether experimental or *in silico*. Our ITC results indicate that the N501Y mutation, found in the Alpha variant, and the E484K mutation, present in the Zeta variant, lead to a 2.20-fold and 5.85-fold increase in binding affinity, respectively. That supports the findings of computational simulations. Importantly, the highest affinity for the hACE2 receptor was observed with the RBM fragment that included both mutations, resulting in an impressive 15-fold increase in affinity.

References

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author email: danuta.witkowska@uni.opole.pl