 doi:10.20944/preprints202209.0318.v1

Article

Last but not Least Important Variant: The Most Negative Gibbs Energy of Binding Indicates the Greatest Infectivity of the BA.2.75 Compared with Other SARS-CoV-2 Variants

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Abstract: Omicron BA.2.75 may become the next globally dominant strain of COVID-19 in 2022. *BA.2.75* sub-*variant* has acquired more mutations (9) in spike protein and other genes of SARS-CoV-2 than any other variant. Thus, its chemical composition and thermodynamic properties have changed comparing to earlier variants. In this paper Gibbs energy of binding and antigen-receptor binding rate is reported for the BA.2.75 variant. Gibbs energy of binding of Omicron BA.2.75 variant is more negative than that of the competing variants BA.2 and BA.5.

Keywords: BA.2.75 variant; Gibbs energy of binding; binding rate; infectivity; SARS-CoV-2

1. Introduction

Multicellular organisms can be considered as open thermodynamic systems exhibiting growth [1-3]. Microorganisms, including viruses, represent open thermodynamic systems with the property of growth through multiplication [4-11]. Microorganisms perform chemical, physical and biological interactions with their environment, other microorganisms and their host [4, 5, 7, 12-16]. The basic condition for virus-host interaction is the presence of an appropriate antigen on the virus and an appropriate receptor on the host cell [12]. The receptor on human cells susceptible to SARS-CoV-2 is angiotensin-converting enzyme 2 (ACE2). SARS-CoV-2 binds to its host cells using its antigen, the spike glycoprotein trimer (SGP) [17, 18].

Microorganisms represent open thermodynamic systems, exchanging matter and energy with their surroundings [2]. Thermodynamic properties are available for more than 50 microorganisms [7]. Thermodynamic analysis has been done of biochemical processes performed by microorganisms [19-24]. Thermodynamic driving force for growth of microorganisms has been analyzed by von Stockar [5, 6, 25]. Application of laws of thermodynamics in biology and medicine is available in the literature [26-31, 60]. Hansen has underlined the importance of calorimetry in life sciences and drew a parallel between biological evolution and the laws of thermodynamics [32-34]. In 2022, data have been published on thermodynamic properties on Monkeypox and Vaccinia viruses [35]. Atom counting method was developed to obtain empirical formula and thermodynamic properties of viruses [36].

SARS-CoV-2 is an RNA virus. RNA mutate more often than DNA viruses [37]. Starting from the original Hu-1 variant, SARS-CoV-2 has developed several dozen mutations [38, 39]. These mutations contributed to increase in infectivity, in accordance with the predictions of theory of evolution [40]. Some mutations contributed to increase in infectivity, while others contributed to immune response evasion [18, 38].

The goal of this paper is to, based on available literature data, calculate the value of standard Gibbs energy of binding of the BA.2.75 variant, as well as to determine the antigen-receptor binding rate. Moreover, using a mechanistic model, an explanation will be made for increase in infectivity of BA.2.75 compared to BA.2 and BA.5, which currently compete during the summer waive of COVID-19, in July and August 2022.

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2. Materials and Methods

Dissociation equilibrium constants for the spike glycoprotein trimer (SGP) of SARS-CoV-2 to the human angiotensin-converting enzyme 2 (ACE2) were taken from Cao et al. [41]. Their values are presented in Table 1. Their measurement was made at 25°C, by surface plasmon resonance [41].

Binding of the virus antigen (SGP) to the host cell receptor (ACE2) represents a chemical reaction. The rate of this chemical reaction is can be calculated using the binding phenomenological equation

$$r_B = -\frac{L_B}{T}\Delta_B G \quad (1)$$

where r_B is the rate of binding of SGP to hACE2, L_B the binding phenomenological coefficient, T temperature and $\Delta_B G$ Gibbs energy of binding of SGP to hACE2 [4, 42-45]. The binding phenomenological equation shows that the rate of binding is proportional to the negative value of the Gibbs energy of binding.

Barton et al. [38] reported that mutations in viruses lead to changes in binding affinity and standard Gibbs energy of binding. Standard Gibbs energy of binding quantifies the strength with which the virus antigen binds to host cell receptor. The strength of antigen-receptor interactions is related to the ability of coronaviruses to infect human hosts [46]. Mutations induce significant changes in SGP conformation [47]. The mutations that lead to higher binding affinity are promoted by evolution through natural selection [47]. The quantitative measure of binding affinity is Gibbs energy of binding [13-16].

Gibbs energy of binding can be determined using dissociation equilibrium constants. Standard Gibbs energy of binding, $\Delta_B G^o$, is given by the equation

$$\Delta_B G^0 = -R_g T \ln(K_B) \quad (2)$$

where R_g is the universal gas constant, T temperature and K_B the binding equilibrium constant [42, 48]. K_B can be found as the reciprocal of the dissociation equilibrium constant, K_D [48].

$$K_B = \frac{1}{K_D} \quad (3)$$

The dissociation equilibrium constant is defined for the dissociation reaction of the antigen-receptor complex.

$$AR \rightleftarrows A + R \quad (4)$$

Where AR represents the antigen-receptor complex, A the virus antigen (SGP), and R the host cell receptor (hACE2) [42, 48]. Thus, K_D is defined through the free antigen concentration $[A]$, free receptor concentration $[R]$ and antigen-receptor complex concentration $[AR]$ [42, 48]

$$K_D = \frac{[A][R]}{[AR]} \quad (5)$$

Gibbs energy of binding was calculated from the binding equilibrium constant, which in turn was found from the dissociation equilibrium constant. $\Delta_B G^o$ is the thermodynamic driving force for the chemical reaction of antigen-receptor binding.

3. Results

Standard Gibbs energies of binding were determined for BA.2.75, BA.2, BA.4/5 and other major SARS-CoV-2 variants. They are given in Table 1. Standard Gibbs energy of binding of BA.2 variant was found to be -45.81 kJ/mol, while for BA.5 it is -44.95 kJ/mol. Finally, for BA.2.75, standard Gibbs energy of binding was found to be -49.91 kJ/mol.

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Table 1. Standard thermodynamic properties of binding of SARS-CoV-2 variants. The table shows association rate constant, k_{on}, dissociation rate constant, k_{off}, dissociation equilibrium constant, K_d, binding phenomenological coefficient, L_B, binding equilibrium constant, K_B, and standard Gibbs energy of binding, $\Delta_B G^0$, data at 25°C. The k_{on}, k_{off} and K_d data were taken from [41].

Name	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_d (M)	L_B (mol^2 K / J s dm^3)	K_B (M^{-1})	$\Delta_B G^0$ (kJ/mol)
BA.2	4.06E+06	3.82E-02	9.40E-09	8.01E-17	1.06E+08	-45.81
BA.4/5	5.30E+05	7.07E-03	1.33E-08	1.48E-17	7.52E+07	-44.95
BA.2.75	1.88E+06	4.22E-03	2.20E-09	8.68E-18	4.55E+08	-49.41
BA.2.75 (Q493R)	8.85E+05	5.64E-03	6.40E-09	1.19E-17	1.56E+08	-46.77
BA.2.75 (S446G)	3.36E+06	1.18E-02	3.50E-09	2.47E-17	2.86E+08	-48.26
BA.2.75 (N460K)	3.87E+07	5.02E-01	1.38E-08	1.12E-15	7.25E+07	-44.86
B.1.1.7 (Alpha)	7.38E+05	3.55E-03	4.80E-09	7.43E-18	2.08E+08	-47.48
B.1.351 (Beta)	5.42E+05	7.31E-03	1.35E-08	1.54E-17	7.41E+07	-44.92
P.1 (Gamma)	3.77E+05	6.29E-03	1.67E-08	1.32E-17	5.99E+07	-44.39
B.1.617.2 (Delta)	7.21E+05	7.84E-03	1.09E-08	1.65E-17	9.17E+07	-45.45
BA.1	1.04E+06	1.07E-02	1.03E-08	2.25E-17	9.71E+07	-45.59
BA.2.12.1	9.08E+05	9.41E-03	1.04E-08	1.98E-17	9.62E+07	-45.56
BA.3	1.54E+06	3.16E-02	2.04E-08	6.59E-17	4.90E+07	-43.89
BA.2.75 (H339)	2.81E+06	6.72E-03	2.40E-09	1.41E-17	4.17E+08	-49.20

Binding rates of the analyzed SARS-CoV-2 variants were calculated and are presented in Table 2. Binding rate for BA.2 variant was found to be $6.58 \cdot 10^{-17}$ M/s, while for BA.5 it is $1.19 \cdot 10^{-17}$ M/s. Finally, for BA.2.75 it is $5.74 \cdot 10^{-18}$ M/s, while for BA.2.75 (N460K) it is $1.49 \cdot 10^{-15}$ M/s.

Binding equilibrium constants of the analyzed SARS-CoV-2 variants were calculated and are shown in Table 1. Binding equilibrium constant of BA.2 variant was found to be $1.06 \cdot 10^{8}$ M/s, while for BA.5 it is $7.52 \cdot 10^{7}$ M/s. Binding equilibrium constant of BA.2.75 variant is $4.55 \cdot 10^{8}$ M/s.

4. Discussion

The direction of development of COVID-19 pandemic depends on two biological properties: infectivity and pathogenicity of SARS-CoV-2 [40]. Infectivity and pathogenicity are biological properties, which are a consequence of virus-host interactions [39, 49]. Virus-host interactions have a chemical and thermodynamic background [9, 13-16, 50-53]. Infectivity depends on the entry rate of the virus into susceptible cells [42]. Pathogenicity depends on the rate of virus multiplication [42]. Virus entry rate is a kinetic property. In its essence, the entry is preceded by antigen-receptor binding. Antigen-receptor binding represents a process similar to protein-ligand interactions [44, 48]. The driving force for antigen-receptor binding is Gibbs energy of binding [13-16, 43]. Since 2019, SARS-CoV-2 has evolved continuously through acquisition of multiple mutations [38]. According to the evolution theory, it is expected that mutations lead towards increase in infectivity and maintenance or decrease in pathogenicity [40]. Virus multiplication represents a chemical process of polymerization of nucleotides and amino acids into virus building blocks [51]. The driving force for virus population growth is Gibbs energy of biosynthesis [40, 44].

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Table 2. Binding rates of SARS-CoV-2 variants. The table shows r_{kin}, r_{TD} and r_{exp}: binding rates calculated using the kinetic, thermodynamic and exponential methods, respectively. The values were calculated at $Q = 0.91\ K_B$.

Name	r_{kin} (M/s)	r_{TD} (M/s)	r_{exp} (M/s)
BA.2	6.58E-17	6.34E-17	6.64E-17
BA.4/5	1.19E-17	1.17E-17	1.23E-17
BA.2.75	5.74E-18	6.88E-18	7.20E-18
BA.2.75 (Q493R)	1.03E-17	9.42E-18	9.86E-18
BA.2.75 (S446G)	1.98E-17	1.95E-17	2.05E-17
BA.2.75 (N460K)	1.49E-15	8.88E-16	9.29E-16
B.1.1.7 (Alpha)	6.03E-18	5.89E-18	6.16E-18
B.1.351 (Beta)	1.29E-17	1.22E-17	1.27E-17
P.1 (Gamma)	1.11E-17	1.05E-17	1.10E-17
B.1.617.2 (Delta)	1.40E-17	1.31E-17	1.37E-17
BA.1	1.88E-17	1.78E-17	1.86E-17
BA.2.12.1	1.70E-17	1.57E-17	1.64E-17
BA.3	5.15E-17	5.22E-17	5.47E-17
BA.2.75 (H339)	1.22E-17	1.12E-17	1.17E-17

In this paper, Gibbs energies of binding were calculated, based on kinetic and thermodynamic properties, k_{on}, k_{off} and K_d, reported by Cao et al. [41] for the currently dominant BA.2.75 Omicron variant. Gibbs energy of binding of BA.2.75 Omicron variant was calculated to be -49.41 kJ/mol (Table 1). BA.2.75, is increasing in frequency, and has been detected in at least 15 countries as end of July, 2022. This means that BA.2.75 is suppressing the existing BA.4 and BA.5 variants. This leads to the conclusion that infectivity of BA.2.75 is greater than that of BA.4 and BA.5. In that case, BA.2.75 is characterized by a more negative Gibbs energy of binding than BA.4 and BA.5. Moreover, the rate of entry into host cells depends on three factors: Gibbs energy of binding, binding phenomenological coefficient and temperature. Temperature at which most biological processes occur is the physiological temperature of 37°C. The calculated binding phenomenological coefficients are given in Table 1. The calculated rates of binding of the viral spike glycoprotein trimer (SGP) to the human angiotensin-converting enzyme 2 (ACE2) are given in Table 2. Relative to the BA.2 variant, BA.2.75 carries 9 additional mutations in the spike glycoprotein [54, 55]. Mutation cause change in elemental composition and empirical formula, leading to change in thermodynamic properties. The underlying mechanism of BA.2.75's enhanced infectivity, especially compared to BA.5, remains unclear for now [41].

Various Omicron strains compete for soil [40, 53]. This means that BA.2.75 competes with BA.2 and BA.5. Since we know that BA.2.75 wins, it is expected to have a more negative Gibbs energy of binding than other variants, as well as greater entry rate and infectivity. Table 1 shows $\Delta_B G^o$ values for several SARS-CoV-2 variants. $\Delta_B G^o$ values of BA.2 and BA.5 variants are -45.81 kJ/mol and -44.95 kJ/mol, respectively. Indeed, $\Delta_B G^o$ of BA.2.75 is more negative than that of competing variants. This observation explains both the greater infectivity and suppression of previous variants by BA.2.75.

The entry rate of SARS-CoV-2 variants was calculated using three approaches: kinetic, thermodynamic and exponential. The kinetic approach uses the law of mass action with k_{on} and k_{off} rate constants [44, 56, 57]. Thermodynamic approach uses the binding phenomenological equation [44, 45, 58]. The exponential approach uses a more general exponential equation from nonequilibrium thermodynamics [44, 45]. The results are shown in Table 2. The entry rates of BA.2 and BA.5 variants were found to be $6.58 \cdot 10^{-17}$ M/s and $1.19 \cdot 10^{-17}$ M/s, respectively. On the other hand, the entry rate of BA.2.75 was found to be $5.74 \cdot 10^{-18}$ M/s, using the kinetic method. This can be explained by a difference in binding phenomenological coefficients, L_B. However, the variant BA.2.75 (N460K) exhibits the

 doi:10.20944/preprints202209.0318.v1

greatest binding rate of $1.49 \cdot 10^{-15}$ M/s. Thus, the binding rate of BA.2.75 (N460K) is 23 times greater than that of BA.2 and 125 times greater than that of BA.5.

The mutations G446S and *N460K* are present in the BA.2.75 variant. They were found to provide the BA.2.75 variant enhanced resistance to neutralizing antibodies [59]. However, it seems that it is not only evasion of immune response, but also more negative Gibbs energy of binding and entry rate into host cells, as shown by results in Table 2.

5. Conclusions

Gibbs energy of binding of Omicron BA.2.75 variant is more negative than that of the competing BA.2 and BA.5. This may be the reason why the BA.2.75 variant has exhibited a high infectivity in India and other countries.

Mutation N460K on BA.2.75 variant contributes not only to evading immune response, but also to faster antigen-receptor binding. Thus, infectivity of this variant is greater than that of competing variants.

The greatest rate of binding to host cell receptors is that of BA.2.75 with the mutation N460K, being 23 times greater than that of BA.2 and 125 times greater than that of BA.5.

Funding: This research received no external funding

Conflicts of Interest: The authors declare no conflict of interest.

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