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Why doesn't Ebola virus cause pandemics like SARS-CoV-2?

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ABSTRACT

Ebola virus is among the most dangerous, contagious and deadly etiological causes of viral diseases. However, Ebola virus has never extensively spread in human population and never have led to a pandemic. Why? The mechanistic biophysical model revealing the biothermodynamic background of virus-host interaction) could help us to understand pathogenesis of Ebola virus disease (earlier known as the Ebola hemorrhagic fever). In this paper for the first time the empirical formula, thermodynamic properties of biosynthesis (including the driving force of virus multiplication in the susceptible host), binding constant and thermodynamic properties of binding are reported. Thermodynamic data for Ebola virus were compared with data for SARS-CoV-2 to explain why SARS-CoV-2 has caused a pandemic, while Ebola remains on local epidemic level. The empirical formula of the Ebola virus was found to be $CH_{1.569}O_{0.3281}N_{0.2786}P_{0.00173}S_{0.00258}$. Standard Gibbs energy of biosynthesis of the Ebola virus nucleocapsid is -151.59 kJ/C-mol.

1. Introduction

In addition to being biological systems, organisms represent chemical and open thermodynamic systems out of equilibrium (von Bertalanffy, 1950; Popovic, 2018, 2017a, 2017b; Lucia and Grisolia, 2020; Lucia, 2015). Microorganisms represent open nonequilibrium thermodynamic systems (von Stockar, 2013a, 2013b; Popovic, 2019; Popovic et al., 2021; Battley, 2013). Viruses also represent open thermodynamic systems (Popovic and Minceva, 2020a, 2020b; Maskow et al., 2010a; Guosheng et al., 2003; Popovic, 2022g), which interact with their host cells and with other viruses (Popovic and Popovic, 2022; Popovic and Minceva, 2021a). Host cells also represent thermodynamic systems (Popovic and Minceva, 2021b, 2020c). Virus-host interactions occur at (a) the cell membrane (antigen receptor binding) (Popovic, 2022a, 2022b) and (b) in the cytoplasm (replication, transcription, translation and self-assembly) (Popovic and Minceva, 2020a). These virus-host interactions are in their essence chemical processes. Antigen-receptor binding represents an interaction similar to protein-ligand binding (Du et al., 2016). On the other hand, transcription and translation represent polymerization processes of nucleotides into nucleic acids and amino acids into proteins (Berg et al., 2002). The driving force for chemical reactions is Gibbs energy (Demirel, 2014; Balmer, 2011). Similarly, the driving force for the binding process is Gibbs energy of binding (Gale, 2021, 2020, 2019, 2018; Popovic, 2022d, 2022e). The driving force for virus multiplication (through replication, transcription, translation and self-assembly) is Gibbs energy of biosynthesis (Popovic, 2022b; Popovic and Minceva, 2020a). Thus, energetics of interactions between microorganisms is of great importance (Mahmoudabadi et al., 2017; Yildiz and Özilgen, 2022; Lucia et al., 2020a).

To explore the energetics of virus-host interactions, it is necessary to find thermodynamic properties of viruses and their host. This is often difficult, since most analytic and thermodynamic laboratories lack the required biosafety level (Popovic, 2022c). Since due to this limitation it is difficult to experimentally determine thermodynamic properties of viruses, atom counting method has been developed (Popovic, 2022c), which allows determining empirical formulas and thermodynamic properties of formation and biosynthesis (growth) (Popovic, 2022c).

The Ebola virus is one of the viruses, for which the elemental composition, and thermodynamic properties of binding and biosynthesis have not been determined. Indeed, experimentally determined elemental composition is available only for the poliovirus (Wimmer, 2006; Molla et al., 1991). However, calculated empirical formulas of 20 viruses and phages are available in the literature (Popovic and Minceva, 2020a, 2020b, 2021a; Popovic, 2022b, 2022c; Degueldre, 2021; Şimşek et al., 2021). The results obtained using the atom counting method are in good agreement with experimental results (Popovic, 2022c). Thermodynamic properties of live matter and biosynthesis of viruses are available in (Popovic and Minceva, 2020a, 2020b, 2021a; Popovic,

"The Ebola war wasn't won with modern medicine. It was a medieval war, and it went down as a brutal engagement between people and a life form that was trying to use the human body as a means of survival." Richard Preston, Crisis in the Red Zone, 2019.

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2022b, 2022c; Şimşek et al., 2021). Thermodynamic properties of binding of viruses to their host cells are available in (Gale, 2021, 2020, 2019, 2018; Popovic and Popovic, 2022; Popovic, 2022a, 2022b, 2022d, 2022e).

The Ebola virus is a single-stranded negative-sense RNA virus. Six subtypes of Ebola virus have been identified: Bundibugyo, Reston, Sudan, Tai forest, Zaire and Bombali. Four of them cause Ebola virus disease (EVD) in humans. EVD causes hemorrhagic fever in humans. Zaire Ebola virus exhibits the highest mortality rate (up to 90% mortality). The virus spreads through direct contact with body fluids. The Ebola virus structural glycoprotein is responsible for the receptor binding. TIM-1 receptor on T-lymphocytes is the place of binding. The interaction between TIM-1 and Ebola GP represents a process similar to protein-ligand binding. The binding rate (kinetic property) depends on Gibbs energy of binding (thermodynamic property) and is given by the binding phenomenological equation, which belongs to nonequilibrium thermodynamics

$$r_B = -\frac{L_B}{T}\Delta_B G \tag{1}$$

where r_B is binding rate, L_B binding phenomenological coefficient, T temperature and $\Delta_B G$ Gibbs energy of binding (Popovic and Popovic, 2022; Popovic, 2022a, 2022b). Thus, it is very important to know the driving force of the antigen-receptor reaction. TIM-1 serves as the receptor for Ebola virus *in vivo* (Brunton et al., 2019). Ebola infection occurs when the virus gains access to a "susceptible host" via a "portal of entry" (skin or mucous membranes) (Shultz, 2016). Susceptibility and permissiveness are two biological properties of virus-host interactions, with a biothermodynamic background. Susceptibility depends on Gibbs energy of binding, while permissiveness depends on Gibbs energy of biosynthesis (growth) (Popovic, 2022b). Both susceptibility and permissiveness of Ebola virus have not yet been described quantitatively using thermodynamic and kinetic parameters.

Research in the field of experimental thermodynamics of organisms have begun during the late 18th century with the works of Lavoisier and Laplace (Lavoisier and Marquis de Laplace, 1783; Müller, 2010). Theoretical application of thermodynamics to study organisms was pioneered by Boltzmann (1974). Morowitz has shed light on controversial questions related to the second law of thermodynamics and properties of biological systems (Morowitz, 1968, 1992, 1955). Schrödinger has contributed to development and popularization of biothermodynamics (Schrödinger, 1944). Prigogine has enabled a more realistic analysis of behavior of organisms, through development of nonequilibrium thermodynamics (Glansdorff and Prigogine, 1971; Prigogine, 1977, 1947; Prigogine and Wiame, 1946). Hansen drew a parallel between the laws of thermodynamics and evolution (Hansen et al., 2009, 2018, 2021). Von Stockar identified Gibbs energy as the driving force for the key property of living organisms – growth (von Stockar, 2013a, 2013b; von Stockar and Liu, 1999; von Stockar et al., 2013, 2006; Liu et al., 2007). Maskow has through his work in experimental biothermodynamics given support for theoretical research (Maskow, 2013; Maskow et al., 2010b; Maskow and von Stockar, 2005). Guosheng et al. (2003) were the first to apply calorimetry to study viruses. Lucia has applied biothermodynamics in research on viruses and epidemiology (Lucia et al., 2021, 2020a, 2020b; Kaniadakis et al., 2020). Thermodynamic analysis has also been applied to biochemical processes performed by microorganisms (Greinert et al., 2020a, 2020b; Meuer et al., 2017, 2016; Wangler et al., 2018; Popovic et al., 2019).

Spreading of a virus during epidemics or pandemics is a complex process, depending on many factors. Biological and thermodynamic properties of a virus are some of them. Spreading of viruses is also influenced by transmission paths, number of potential doors of entry and specific receptors, communication between populations, anti-epidemic measures etc. (Riedel et al., 2019). In this paper, a mechanistic model will be presented that will cover only the biothermodynamic and bioenergetic aspects of virus-host interactions. Thus, it represents a mechanistic model that considers virus-host interactions at the molecular level. The influence of the macroscopic parameters mentioned above (epidemiological and sociological) has not been taken into account into this study. It is certain that common and close contacts of host organisms and absence of anti-epidemic measures leads to greater likelihood of infection, due to greater concentration of viruses in some environments (e.g. small, closed and badly ventilated spaces). A high concentration of viruses in such spaces can easily reach the inoculum size required for beginning an infection (Asabe et al., 2009).

The influence of virus concentration on Gibbs energy of binding can be described by the equation

$$\Delta_B G = \Delta_B G^0 + R_g T \ln Q \tag{2}$$

where $\Delta_B G$ is Gibbs energy of binding, $\Delta_B G^o$ standard Gibbs energy of binding, R_g universal gas constant and T temperature (Popovic, 2022h). The parameter Q is the reaction quotient, describing the influence of virus concentration on Gibbs energy of binding (Popovic, 2022h).

$$Q = \frac{[AR]}{[A][R]} \tag{3}$$

where $[A]$ is the concentration of the free virus antigen, $[R]$ the concentration of the free host cell receptor and $[AR]$ the concentration of the antigen-receptor complex (Popovic, 2022h). The greater the concentration of virus particles in the inoculum, the greater the virus antigen concentration $[A]$. Greater $[A]$ value makes Q smaller in Eq. (3), which in turn makes $\Delta_B G$ more negative in Eq. (2). Thus, the biophysical model is able to take into account the inoculum size as a correction to Gibbs energy of binding. The dependence of inoculum size on the epidemiological and sociological factors described above would take a lot of additional calculations and is thus beyond the scope of this research. However, it would be an interesting subject for future research. Thus, the proposed model covers the biophysical aspect of virus-host interactions, at the molecular level. However, infection is dynamic and involves both host, environmental, host genetic, nutritional, microbiome and other aspects. Thus, the proposed model can also be included into wider models, due to its simplicity.

The aim of this paper is to determine thermodynamic properties, binding constants and Gibbs energies of binding for the Ebola virus, which represents the physicochemical background of susceptibility. Moreover, empirical formula and Gibbs energy of biosynthesis should also be determined for the Ebola virus.

2. Methods

2.1. Data sources

The genetic and protein sequences of the Ebola virus were obtained from the NCBI database [National Center for Biotechnology Information, 2022). The genetic sequence of the Ebola virus was found under the acquisition number KY786026.1. The nucleoprotein was found under the acquisition number O72142.1. Protein VP24 was found under the acquisition number Q6V1Q3.1. Protein VP30 was found under the acquisition number Q77DJ5.1. Protein VP35 was found under the acquisition number Q6V1Q9.1. Protein copy numbers in the Ebola virus nucleocapsid were taken from Beniac et al. (2012). The dissociation constants of the Ebola virus subtypes were taken from Yuan et al. (2015).

2.2. Elemental composition of viral nucleocapsid

Elemental composition of virus nucleocapsid was calculated, using the atom counting method (Popovic, 2022c). The atom counting method calculates the number of atoms of each element in a virus particle or its part, based on its genetic sequence, protein sequences, protein copy numbers and virus size (Popovic, 2022c). The elemental composition of

the virus nucleocapsid was determined from genetic sequences, protein sequences and protein copy numbers. The atom counting method was applied, using a custom-made computer program. More details on the atom counting method can be found in Popovic (2022c).

2.3. Standard thermodynamic properties of live matter

Elemental composition of live matter was used to find standard thermodynamic properties of the Ebola virus nucleocapsid. Standard enthalpy of formation of virus live matter was calculated using the Patel-Erickson equation, also known as Thornton's rule. Elemental composition of live matter can be used to find the number of electrons transferred to oxygen during its complete combustion, E, using the equation

$$E = 4n_C + n_H - 2n_O - 0\,n_N + 5n_P + 6n_S \tag{4}$$

where n_C, n_H, n_O, n_N, n_P and n_S represent the number of carbon, hydrogen, oxygen, nitrogen, phosphorus and sulfur atoms in the empirical formula of live matter, respectively (Battley, 1998, 1992; Popovic, 2022b, 2019). The number of electrons E can be used to calculate standard enthalpy of combustion of live matter, $\Delta_C H^\circ(bio)$, using the Patel-Erickson equation (Patel and Erickson, 1981; Battley, 1998, 1992; Popovic, 2022b, 2019)

$$\Delta_C H^0(bio) = -111.14\frac{kJ}{C-mol}\cdot E \tag{5}$$

$\Delta_C H^\circ(bio)$ is the enthalpy change of the combustion reaction of live matter

$$C_{nC}H_{nH}O_{nO}N_{nN}P_{nP}S_{nS} + (n_C + 1/4n_H + 11/4n_P + 11/2n_S - 1/2n_O)O_2 \rightarrow n_C CO_2 + 1/2n_H H_2O + 1/2n_N N_2 + 1/4n_P P_4O_{10} + n_S SO_3 \tag{6}$$

Thus, Hess's law can be used to convert standard enthalpy of combustion of live matter, $\Delta_C H^\circ(bio)$, into standard enthalpy of formation of live matter, $\Delta_f H^\circ(bio)$ (Atkins and de Paula, 2011, 2014).

$$\Delta_f H^0(bio) = n_C\Delta_f H^0(CO_2) + \frac{n_H}{2}\Delta_f H^0(H_2O) + \frac{n_P}{4}\Delta_f H^0(P_4O_{10}) + n_S\Delta_f H^0(SO_3) - \Delta_C H^0(bio) \tag{7}$$

A similar procedure can be used to find standard molar entropy of virus live matter, using the Battley equation. The Battley equation relates elemental composition of live matter to its standard molar entropy, $S_m^0(bio)$

$$S_m^0(bio) = 0.187\sum_J \frac{S_m^0(J)}{a_J}n_J \tag{8}$$

where $S_m^0(J)$ is standard molar entropy of element J, a_J number of atoms of element J in its standard state form and n_J is the number of atoms of element J in the empirical formula of the virus (Battley, 1999; Popovic, 2022b, 2019). The Battley equation can be modified to give standard entropy of formation of live matter, $\Delta_f S^\circ(bio)$. This is done by replacing the coefficient +0.187 with -0.813 (Battley, 1999)

$$\Delta_f S^0(bio) = -0.813\sum_J \frac{S_m^0(J)}{a_J}n_J \tag{9}$$

Finally, $\Delta_f S^\circ(bio)$ can be combined with $\Delta_f H^\circ(bio)$, to find standard Gibbs energy of formation of live matter, $\Delta_f G^\circ(bio)$, using the equation (Battley, 1998; Popovic, 2022b, 2019)

$$\Delta_f G^0(bio) = \Delta_f H^0(bio) - T\Delta_f S^0(bio) \tag{10}$$

2.4. Biosynthesis reactions

Elemental composition of virus particles was used to construct biosynthesis reactions of the Ebola virus nucleocapsids. Biosynthesis reactions are macrochemical equations that quantify growth of organisms, describing conversion of nutrients into new live matter and other metabolic products (von Stockar, 2013a, 2013b; Battley, 1998, 2013). Biosynthesis reactions have been used to study a wide range of organisms, including bacteria (Battley, 1992), fungi (Battley, 2013, 1998), algae (Wang et al., 2017), plants (Popovic and Minceva, 2021b) and viruses (Popovic and Minceva, 2020a, 2020b, 2021a). Biosynthesis reactions for the analyzed viruses have the general form (Popovic, 2022b; Popovic and Minceva, 2020a, 2020b, 2021a)

$$(\text{Aminoacid}) + O_2 + HPO_4^{2-} + HCO_3^- \rightarrow (\text{Bio}) + SO_4^{2-} + H_2O + H_2CO_3 \tag{11}$$

Amino acids represent the carbon and energy source, and the nitrogen source (Popovic, 2022b; Popovic and Minceva, 2020a, 2020b; von Stockar, 2013b). Oxygen is the electron acceptor (Popovic, 2022b; Popovic and Minceva, 2020a, 2020b; von Stockar, 2013b). The hydrogenphosphate ion is the phosphorus source, while the hydrogencarbonate ion is a part of the bicarbonate buffer that takes the produced H^+ ions (Popovic, 2022b; Popovic and Minceva, 2020a, 2020b; von Stockar, 2013b). (Bio) denotes newly synthetized virus live matter (Popovic, 2022b; Popovic and Minceva, 2020a, 2020b; von Stockar, 2013b). The sulfate ion takes excess sulfur, while H_2CO_3 takes oxidized carbon and excess H^+ ions, as a part of the bicarbonate buffer (Popovic, 2022b; Popovic and Minceva, 2020a, 2020b; von Stockar, 2013b). The stoichiometric coefficients for the biosynthesis reactions of the analyzed viruses are given in Table 5.

2.5. Standard thermodynamic properties of biosynthesis

Standard thermodynamic properties of virus live matter can be combined with biosynthesis reactions, to find standard thermodynamic properties of biosynthesis (von Stockar, 2013a, 2013b; Popovic, 2022b). Standard thermodynamic properties of biosynthesis are thermodynamic property changes accompanying biosynthesis reactions (von Stockar, 2013a, 2013b; Popovic, 2022b). They include standard enthalpy of biosynthesis, $\Delta_{bs}H^\circ$, standard entropy of biosynthesis, $\Delta_{bs}S^\circ$, and standard Gibbs energy of biosynthesis, $\Delta_{bs}G^\circ$ (von Stockar, 2013a, 2013b; Popovic, 2022b). These properties can be found using the Hess's law

$$\Delta_{bs}H^0 = \sum_{products}\nu\Delta_f H^0 - \sum_{reactants}\nu\Delta_f H^0 \tag{12}$$

$$\Delta_{bs}S^0 = \sum_{products}\nu S_m^o - \sum_{reactants}\nu S_m^o \tag{13}$$

$$\Delta_{bs}G^0 = \sum_{products}\nu\Delta_f G^0 - \sum_{reactants}\nu\Delta_f G^0 \tag{14}$$

where ν represents a stoichiometric coefficient (Atkins and de Paula, 2011, 2014). Standard Gibbs energy of biosynthesis, $\Delta_{bs}G^\circ$, is of particular importance, since it represents the driving force of biosynthesis and is related to biosynthesis rate (von Stockar, 2013a, 2013b; Popovic, 2022b).

2.6. Standard thermodynamic properties of binding

The dissociation process is the opposite of binding (Du et al., 2016;

Popovic, 2022b). Thus, dissociation equilibrium constants are reciprocal of binding equilibrium constants (Du et al., 2016; Popovic, 2022b). Thus, dissociation equilibrium constants were used to calculate binding equilibrium constants, K_B, using the equation (Du et al., 2016; Popovic, 2022b)

$$K_B = \frac{1}{K_D} \tag{15}$$

The binding constants were used to find standard Gibbs energy of binding, $\Delta_B G^o$, using the equation (Du et al., 2016; Popovic, 2022b)

$$\Delta_B G^0 = -R_s T \ln K_B \tag{16}$$

3. Results

Binding equilibrium constants, K_B, have been determined for Ebola virus strains. They are shown in Table 1. The K_B of the Bundibugyo GP (1-308) to hTIM-1 IgV domain is $2.03 \bullet 10^5$ M. The K_B of the Zaire GP (1-308) to hTIM-1 IgV domain is $8.33 \bullet 10^4$ M. The binding equilibrium constant of Zaire GP (1-320) to hTIM-1 IgV domain is $3.75 \bullet 10^4$ M. The K_B of Zaire GP (1-501) to hTIM-1 IgV domain is $5.29 \bullet 10^5$ M. The binding equilibrium constant of Deglycosilated Zaire GP (1-320) to hTIM-1 IgV domain is $2.06 \bullet 10^4$ M.

Standard Gibbs energies of binding, $\Delta_B G^o$, have been determined for Ebola virus strains. The $\Delta_B G^o$ of Bundibugyo GP (1-308) to hTIM-1 IgV domain is -30.29 kJ/mol. The $\Delta_B G^o$ of Zaire GP (1-308) to hTIM-1 IgV domain is -28.09 kJ/mol. Standard Gibbs energy of binding of Zaire GP (1-320) to hTIM-1 IgV domain is -26.10 kJ/mol. The $\Delta_B G^o$ of Zaire GP (1-501) to hTIM-1 IgV domain is -32.67 kJ/mol. Standard Gibbs energy of binding of Deglycosilated Zaire GP (1-320) to hTIM-1 IgV domain is -24.62 kJ/mol.

The empirical formula of the Ebola virus nucleocapsid is $CH_{1.569}O_{0.3281}N_{0.2786}P_{0.00173}S_{0.00258}$(Table 2). Standard Gibbs energy of biosynthesis is -151.59 kJ/C-mol.

4. Discussion

In this paper, we will analyze virus-host interactions of two different RNA viruses, namely Ebola and SARS-CoV-2. Ebola is transmitted through inhalation of aerosol particles or hand to eye contact, while SARS-CoV-2 is transmitted by aerosol particles (Brunton et al., 2019; Kondratowicz et al., 2011; Gale, 2021; Popovic and Minceva, 2021a). Ebola uses TIM-1 receptor for binding to susceptible host cells (Brunton et al., 2019; Kondratowicz et al., 2011). Kondratowicz et al. (2011) found that recognition that TIM-1 serves as a receptor for filoviruses (including Ebola and Marburg viruses) on the mucosal epithelial surfaces provides a mechanistic understanding of roots of entry into the human body via inhalation of aerosol particles or hand to eye contact. SARS-CoV-2 uses the ACE2 receptor (Gale, 2021; Popovic and Minceva, 2021a). The binding mechanism of the antigen to the receptor is the same – similar to protein-ligand interactions, although the receptors are different. In that case, the binding rate to the receptor and entry of the virus into host cells is driven by Gibbs energy of binding (Popovic, 2022a).

More negative Gibbs energy of binding, according to Eq. (1), implies faster binding reaction and faster virus entry into host cells. The consequence of this is faster spreading of the infection onto a greater population, assuming that the inoculum size is identical. The entry point for both viruses is mucosa, which is susceptible (possesses appropriate receptors).

SARS-CoV-2 spreads much faster than Ebola. Thus, SARS-CoV-2 has caused a pandemic with 600 million registered cases and 6.5 million deaths, during 3 years in practically all countries of the world (Worldometer, 2022; WHO, 2022a). However, Ebola appeared in around 27 000 cases, with a total of 11 000 deaths. The Ebola virus is exhibiting a lower potential for spreading. EVD has in July 2022 appeared as an epidemic in Mbandaka and Vangata, in Congo (WHO, 2022b). The case fatality ratio is 100%. Elucidation of the mechanistic determinants of the outcome of host–Ebola interaction has historically been challenging (Jacob et al., 2020). A single, unified picture of the host–Ebola interaction does not exist (Jacob et al., 2020). Instead, from a 'patchwork' compilation of different and complex observations, key aspects of the human-Ebola interaction remain unknown (Jacob et al., 2020).

Gibbs energy of binding of the spike protein to the ACE2 receptor is available in the literature (Popovic and Popovic, 2022; Popovic, 2022a, 2022b). For the wild type SARS-CoV-2 (Hu-1) standard Gibbs energy of binding was found to be -43.43 kJ/mol, while for the Delta strain it is -43.38 kJ/mol (Popovic, 2022b). In this paper, standard Gibbs energies are reported for the Zaire GP to TIM-1 (Table 1). For Zaire GP (1-308) to hTIM-1 IgV domain $\Delta_b G^o$ is -28.09 kJ/mol. For Zaire GP (1-320) to hTIM-1 IgV domain $\Delta_b G^o$ is -26.10 kJ/mol. For Zaire GP (1-501) to hTIM-1 IgV domain $\Delta_b G^o$ is -32.67 kJ/mol. For Deglycosilated Zaire GP (1-320) to hTIM-1 IgV domain $\Delta_b G^o$ is -24.62 kJ/mol. For Bundibugyo GP (1-308) to hTIM-1 IgV domain $\Delta_b G^o$ is -30.29 kJ/mol. Thus, a difference exists in the values of Gibbs energies of binding of various strains of SARS-CoV-2 and subtypes of the Ebola virus. The difference between Gibbs energies of binding of SARS-CoV-2 and Ebola virus is between 10 and 20 kJ/mol. The difference in thermodynamic parameters leads to difference in binding rate (kinetic parameter), according to Eq. (1). Thus, SARS-CoV-2 in upper respiratory pathways, binding to ACE2, enters at a greater rate than the Ebola virus. The greater rate of entry is beneficial to spreading of the infection. This is evidenced by the fact that new strains of SARS-CoV-2 exhibit a tendency towards decreasing standard Gibbs energy of binding (Popovic and Popovic, 2022; Popovic, 2022f). This coincides with the epidemiological observations that newer strains spread more rapidly, causing greater peaks in the epidemiological curves (Worldometer, 2022; WHO, 2022a). A significantly more negative Gibbs energy of binding leads to slower entry and makes transmission from human to human more difficult. This makes the spreading of the epidemic more difficult. The final result is several times lower number of infections and greater confinement of the epidemics of Ebola, even though Ebola has been present in the human population for over half a century.

Table 1
Dissociation equilibrium constants, K_D, binding equilibrium constants, K_B, and standard Gibbs energies of binding, $\Delta_B G^o$, of Ebola virus strains. All the data are at 25°C. The K_D data were taken from Yuan et al. (2015).

Virus	Strain	K_D (M)	K_B (M^{-1})	$\Delta_b G^o$ (kJ/mol)	Refs.
Ebola virus	Bundibugyo GP (1-308) to hTIM-1 IgV domain	4.93E-06	2.03E+05	-30.29	Yuan et al. (2015)
Ebola virus	Zaire GP (1-308) to hTIM-1 IgV domain	1.20E-05	8.33E+04	-28.09	Yuan et al. (2015)
Ebola virus	Zaire GP (1-320) to hTIM-1 IgV domain	2.67E-05	3.75E+04	-26.10	Yuan et al. (2015)
Ebola virus	Zaire GP (1-501) to hTIM-1 IgV domain	1.89E-06	5.29E+05	-32.67	Yuan et al. (2015)
Ebola virus	Deglycosilated Zaire GP (1-320) to hTIM-1 IgV domain	4.85E-05	2.06E+04	-24.62	Yuan et al. (2015)

Table 2
Empirical formula of the Ebola virus nucleocapsid. n_J denotes the number of atoms of element J in the empirical formula.

Organism	n_C	n_H	n_O	n_N	n_P	n_S
Ebola virus (nucleocapsid)	1	1.569	0.3281	0.2786	0.00173	0.00258

Differences are obvious between Gibbs energies of binding of Zaire GP (1-320) and Zaire GP (1-501), as well as between Bundibugyo and Zaire (1-501). The variant with more negative Gibbs energy of binding should enter host cells faster and transmit more easily.

We can compare the binding constant of SARS-CoV-2 available in the literature (Popovic, 2022b), which is 4.06 •10^7, with the K_B of the Ebola virus reported for the first time in this paper. The K_B of Bundibugyo GP (1-308) to hTIM-1 IgV domain is 2.03 • 10^5 M^{-1}. The K_B of Zaire GP (1-308) to hTIM-1 IgV domain is 8.33 • 10^4 M^{-1}. The K_B of Zaire GP (1-320) to hTIM-1 IgV domain is 3.75 • 10^4 M^{-1}. We can notice that the affinity for binding of ACE2 to SGP (spike glycoprotein) of SARS-CoV-2 is 1000 times greater than the affinity of Ebola GP to TIM-1. From this we can conclude that the susceptibility of mucosa in the upper respiratory pathways is much greater for SARS-CoV-2 than for the Ebola virus. This enables faster entry of the virus into the host cell, faster transmission of the virus between humans, leading to more extensive spreading and a pandemic.

Viruses are specifically characterized by a characteristic morphology and chemical composition (Wimmer, 2006; Molla, 1991; Degueldre, 2021; Popovic and Minceva, 2020a, 2020b, 2021a; Popovic and Popovic, 2022; Popovic, 2022b, 2022c; Şimşek, 2021). Thus, viruses represent open thermodynamic systems that interact with their environment, a susceptible host, as well as with other viruses (Popovic and Minceva, 2021a). All these interactions have a biological, chemical and thermodynamic background. Knowing the thermodynamic background is very important (Head et al., 2022). For more than 25 viruses, thermodynamic properties of binding and biosynthesis are known (Popovic and Minceva, 2020a,2020b, 2021a; Popovic and Popovic, 2022; Popovic, 2022a, 2022b, 2022c). Thus, thermodynamic characterization has been made mostly for human viruses and some phages. Thermodynamic properties of the human host are available in the literature (Popovic and Minceva, 2020c). Based on the available thermodynamic properties of viruses and hosts, we will make a thermodynamic analysis of virus-host interactions.

Virus-host interactions occur at the cell membrane (antigen-receptor binding) and in the cytoplasm (multiplication). Biosynthesis of virus components (nucleic acids, proteins) represent a chemical reaction of polymerization. The driving force for the polymerization reaction is Gibbs energy of biosynthesis (Popovic and Minceva, 2020a). The rate of virus biosynthesis depends on Gibbs energy of biosynthesis, through the phenomenological equation, which belongs to nonequilibrium thermodynamics.

$$r_{bs} = -\frac{L_{bs}}{T}\Delta_{bs}G \tag{17}$$

where r_{bs} is biosynthesis rate, L_{bs} biosynthesis phenomenological coefficient, T temperature and $\Delta_{bs}G$ Gibbs energy of biosynthesis. The more negative Gibbs energy of biosynthesis of viruses leads to greater rate of synthesis of nucleic acids and proteins. The greater biosynthesis rate leads to greater concentration of virus components in the cytoplasm. This leads to faster self-assembly into new virions. The greater number of newly formed virions leads to faster release of a greater amount of new virions and more damage done to the cell and tissue.

Gibbs energy of biosynthesis of SARS-CoV-2 for the Hu-1 strain is -222.2 kJ/C-mol (Popovic and Minceva, 2022b). Gibbs energy of biosynthesis of the nucleocapsid of the Ebola virus is given in Table 4. It is -151.59 kJ/C-mol. Based on Eq. (2), it is possible to conclude that from the kinetic perspective, the biosynthesis rate of SARS-CoV-2 is much greater than that of the Ebola virus.

If the biosynthesis reaction is competitive (there is one metabolic machinery and shared building blocks, for example nucleotides or amino acids), then in case of simultaneous infection with Ebola and SARS-CoV- 2 viruses, due to a great difference in biosynthesis rates, interference can be expected. This is based on the fact that the SARS-CoV-2 enters the cell and multiplies faster, since it has more negative Gibbs energies of binding and biosynthesis (Popovic and Minceva, 2020a). This gives it an advantage over Ebola (Popovic and Minceva, 2020a).

Table 3
Standard thermodynamic properties of formation of the Ebola virus nucleocapsid. $\Delta_f H^o$ denotes standard enthalpy of formation, $S_m{}^o$ standard molar entropy and $\Delta_f G^o$ standard Gibbs energy of formation.

Organism	$\Delta_f H^o$ (kJ/C-mol)	$S^o{}_m$ (J/C-mol K)	$\Delta_f G^o$ (kJ/C-mol)
Ebola virus (nucleocapsid)	-68.36	31.62	-27.37

Standard thermodynamic properties of formation of Ebola virus nucleocapsid are given in Table 3. Standard enthalpy of formation of Ebola virus nucleocapsid was found to be -68.36 kJ/C-mol, while its standard molar entropy is 31.62 J/C-mol K. Standard enthalpy of formation is negative, due to oxidation of less electronegative elements by oxygen and nitrogen (Popovic, 2019). Standard molar entropy is positive in accordance with the third law of thermodynamics (Atkins and de Paula, 2011, 2014). Standard Gibbs energy of formation of the Ebola virus nucleocapsid is -27.37 kJ/C-mol. The negative Gibbs energy of formation indicates that the nucleocapsid live matter does not possess a high energy content relative to elements.

Elemental composition of the Ebola virus nucleocapsid was used to find its biosynthesis reaction, which is given in Table 5. The biosynthesis reaction was combined with standard thermodynamic properties of the Ebola virus nucleocapsid (Table 3) to find standard thermodynamic properties of biosynthesis of the Ebola virus nucleocapsid. Standard thermodynamic properties of biosynthesis of Ebola nucleocapsid are given in Table 4. Standard enthalpy of biosynthesis of Ebola virus nucleocapsid was found to be -158.52 kJ/C-mol. The negative sign indicates that the biosynthesis process is exothermic, which is the case for most microorganisms (von Stockar, 2013a, 2013b; von Stockar and Liu, 1999). Standard entropy of biosynthesis of Ebola nucleocapsid was found to be -23.21 J/C-mol K. The entropy is slightly negative, due to formation of viral polymers like nucleic acids and proteins from nucleotides and amino acids. Standard Gibbs energy of biosynthesis of Ebola nucleocapsid was found to be -151.59 kJ/C-mol. The negative Gibbs energy of biosynthesis represents the thermodynamic driving force for virus multiplication.

To determine the permissiveness of human cells to the Ebola virus, it is necessary to compare the rates of synthesis of viral components with those of host cells. This can be done using permissiveness coefficients (Popovic and Minceva, 2020a, 2020b). The permissiveness coefficient, P, is given by the equation

$$P = \frac{r_{bs}(virus)}{r_{bs}(host)} = \frac{\Delta_{bs}G^0(virus)}{\Delta_{bs}G^0(host)} \tag{18}$$

where $r_{bs}(virus)$ is the virus biosynthesis rate, $r_{bs}(host)$ host biosynthesis rate, $\Delta_{bs}G^o(virus)$ standard Gibbs energy of biosynthesis of the virus, and $\Delta_{bs}G^o(host)$ standard Gibbs energy of biosynthesis of the host (Popovic and Minceva, 2020a, 2020b).

Ebola targets specific cell types, including liver cells, endothelial cells, kidneys and spleen (Falasca et al., 2015). The port of entry for the Ebola virus is eye mucosa. Primary virus multiplication occurs at the

Table 4
Standard thermodynamic properties of biosynthesis of Ebola virus nucleocapsid. $\Delta_{bs}H^o$ denotes standard enthalpy of biosynthesis, $\Delta_{bs}S^o$ standard entropy of biosynthesis and $\Delta_{bs}G^o$ standard Gibbs energy of biosynthesis.

Organism	$\Delta_{bs}H^o$ (kJ/C-mol)	$\Delta_{bs}S^o$ (J/C-mol K)	$\Delta_{bs}G^o$ (kJ/C-mol)
Ebola virus (nucleocapsid)	-158.52	-23.21	-151.59

Table 5
Stoichiometric coefficients for the biosynthesis reaction of the Ebola virus nucleocapsid.

Reactants					Products			
Amino acid	O_2	HPO_4^{2-}	HCO_3^-	→	Bio	SO_4^{2-}	H_2O	H_2CO_3
1.2733	0.3329	0.0016	0.0394	→	1	0.0213	0.0632	0.3127

Table 6
Stoichiometric coefficients for biosynthesis reactions of the human host tissues.

Name	Reactants										Products			
	Amino acid	CH_2O	O_2	HPO_4^{2-}	HCO_3^-	Na^+	K^+	Ca^{2+}	Cl^-	→	Bio	SO_4^{2-}	H_2O	H_2CO_3
Aorta	1.0902	0.0000	0.0856	0.0106	0.0381	0.0071	0.0021	0.0082	0.0000	→	1	0.0169	0.1103	0.1284
Kidney	0.9720	0.0968	0.0000	0.0073	0.0298	0.0099	0.0058	0.0028	0.0064	→	1	0.0148	0.0722	0.0986
Liver	0.9994	0.0248	0.0000	0.0092	0.0188	0.0083	0.0073	0.0000	0.0054	→	1	0.0135	0.0902	0.0430
Spleen	1.0806	0.0000	0.0447	0.0103	0.0215	0.0046	0.0082	0.0000	0.0060	→	1	0.0177	0.0649	0.1021
Lung - parenchyma	1.1266	0.0000	0.1070	0.0074	0.0206	0.0100	0.0059	0.0000	0.0097	→	1	0.0146	0.0661	0.1472

Table 7
Standard thermodynamic properties of biosynthesis of the human host tissues.

Organism	$\Delta_{bs}H°$ (kJ/C-mol)	$\Delta_{bs}S°$ (J/C-mol K)	$\Delta_{bs}G°$ (kJ/C-mol)
Aorta	-42.41	1.08	-42.85
Kidney	-4.60	11.13	-7.91
Liver	-1.40	5.41	-3.10
Spleen	-22.74	3.10	-23.78
Lung - parenchyma	-50.51	-2.80	-49.76

Table 8
Permissiveness coefficients of the Ebola virus and human host tissues. The permissiveness coefficients were calculated as the ratio of Gibbs energies of biosynthesis of the virus and its host tissues. A permissiveness coefficient greater than 1 means that a virus is able to hijack the host cell metabolism. The greater the permissiveness coefficient, the greater the potential of the virus to damage the tissue.

Tissue	P
Aorta	3.5
Kidney	19.2
Liver	48.8
Spleen	6.4
Lung - parenchyma	3.0

port of entry. Elemental composition and standard thermodynamic properties of human tissues are reported in the literature (Popovic and Minceva, 2022c). Based on these data, biosynthesis reactions were formulated and thermodynamic properties of biosynthesis found for human host tissues, including aorta, kidney, liver, spleen and lung. Stoichiometric coefficients for the biosynthesis reactions are given in Table 6. Standard thermodynamic properties of biosynthesis are given in Table 7. Furthermore, permissiveness coefficients have been calculated for the Ebola virus in the host tissues. They are given in Table 8.

Biosynthesis of structural elements of the virus and its host cells are competitive reactions of polymerization, where the virus and host cell compete for resources (e.g. nucleotides, amino acids etc.). Thus, there are two possible reactions of polymerization after infection (amino acids into cellular or viral proteins, respectively). Moreover, replication reactions are competitive, where the cell and virus compete for limited amount of nucleotides. The reaction characterized by more negative Gibbs energy, according to the phenomenological equation, occurs at a greater rate. The faster reaction leads to suppression of the slower reaction. In general, all viruses (including Ebola and SARS-CoV-2) have more negative Gibbs energy of biosynthesis, allowing them to stop the normal metabolic pathways of the host cell and hijack the cell's metabolism.

The permissiveness coefficient represents a ratio of the rates of biosynthesis of structural elements of the virus and host cell. If the permissiveness coefficient is equal to unity, then the rates of biosynthesis of the components of the virus and host cell are equal and the virus will not be able to significantly multiply, accumulate and damage the host cell. On the other hand, if the permissiveness coefficient were lower than 1, the virus would not be able to multiply in the cell at all. However, Gibbs energies of biosynthesis of all the viruses analyzed until now is more negative than that of host cells. This implies that the biosynthesis rate of the virus will be greater than that of the host cell and that the permissiveness coefficient will be greater than 1. The greater the permissiveness coefficient, the greater the rate of synthesis of virus components, meaning that dynamics of virus accumulation inside the cell leads to cell damage faster.

In the case of Ebola virus, the most sensitive are the liver cells, since inside them biosynthesis of virus components occurs at the greatest rate. Inside kidneys, the virus is also able to multiply at a great rate. From Table 8, during an Ebola infection, great damage to liver and kidneys can be expected, according to the model proposed in this paper. This is in agreement with the results of Falasca et al. (2015). However, the permissiveness coefficient is greater than 1 for other tissues as well. Thus, the damages to other tissues can occur during infections with the Ebola virus. However, permissiveness is not the only parameter that influences infections. As mentioned above, susceptibility represents a condition required for a virus to enter a cell. Only after entry, the virus is able to hijack the host cell metabolism and multiply inside the host.

A permissiveness coefficient greater than 1 leads to the conclusion that the biosynthesis rate of the virus will be greater than that of the host tissue. Since the chemical reaction is competitive, this indicates that the synthesis of virus components will have a priority. A greater permissiveness coefficient leads to faster virus multiplication inside a susceptible host, implying greater tissue damage. Indeed, the susceptibility coefficient for the blood vessels was found to be 3.5. This is confirmed by Ebola belonging to hemorrhagic fevers. The damage to the endothel of the blood vessels can be explained by the great permissiveness coefficient. Ebola also damages other organs. The permissiveness coefficient for the kidneys is extremely great. This means that damage to the kidney tissues during EVD will be very pronounced. The permissiveness coefficient for the kidneys is 48.8. A slightly lower permissiveness coefficient is that of liver, 19.2. This leads to the conclusion that multiplication of the Ebola virus in hepathocytes is very fast, leading to extensive damage to liver tissue. Respiratory epithelium also exhibits a great level of permissiveness for the Ebola virus, 3.0. This means that at the entry point the multiplication of the Ebola virus is very intense, allowing virus dissemination into other susceptible tissues.

SARS-CoV-2 enters the host cell by binding to the ACE2 receptor present on most of the human host cells. On the other hand, Ebola has a wide tropism, most likely with multiple receptors and co-receptors, and

alternative entry mechanisms. Antigen of the Ebola virus is the Ebola virus glycoprotein (EVGP). Several cell surface receptors have been identified allowing Ebola virus binding and internalization. One of these is the phosphatidylserine receptor (TIM-1), while the second is C-type lecithin receptor (DC-SIGNR). Zhang et al. (2022) confirmed that both receptors specifically bind to EVGP with high affinity.

Moreover, Ebola virus uses its glycoprotein (EVGP) to enter new host cells. During entry, EVGP must be cleaved by human enzymes in order for receptor binding to occur (Bornholdt et al., 2016). EVGP is cleaved by host cysteine proteases to expose a receptor-binding site (RBS) enabling infection (Bornholdt et al., 2016). To infect cells, Ebolaviruses are internalized via macropinocytosis and traffic through the endosomal pathway where host cathepsin-dependent cleavage of the viral glycoproteins occurs (Bornholdt et al., 2016). Subsequently, the cleaved viral glycoprotein interacts with the late endosome resident host protein, Niemann-Pick C1 (NPC1) (White and Whittaker, 2016; Bornholdt et al., 2016). Thus, even though alternative mechanisms exist for virus entry into host cells, in the literature during a comprehensive search, no kinetic data (k_{on}, k_{off}, K_D) were found to quantify the kinetic aspect of the alternative antigen-receptor interaction, which would enable to calculate thermodynamic properties of these interactions. The author hopes the research can be continued, once the data are available in the literature. This will certainly require the development of a new model, but will also enable insight into the energetics of Ebolavirus-host interactions. Thus, it seems obvious that the progress in the field of kinetics of interactions of Ebolavirus-host interactions could open the path for further biothermodynamic analysis.

5. Conclusions

The empirical formula of the Ebola virus was found to be $CH_{1.569}O_{0.3281}N_{0.2786}P_{0.00173}S_{0.00258}$. Standard Gibbs energy of biosynthesis of the Ebola virus nucleocapsid is -151.59 kJ/C-mol.

Every virus has a characteristic empirical formula. The empirical formula of the Ebola virus is $CH_{1.569}O_{0.3281}N_{0.2786}P_{0.00173}S_{0.00258}$. The driving force for multiplication of viruses is Gibbs energy of biosynthesis for the nucleocapsid. For Ebola virus nucleocapsid, Gibbs energy of biosynthesis is -151.59 kJ/C-mol.

The mechanistic model of antigen-receptor interaction has enabled calculating the driving force for the Ebola virus GP binding to TIM-1. Standard Gibbs energy of binding of Bundibugyo GP (1-308) to hTIM-1 IgV domain is -30.29 kJ/mol. $\Delta_B G^o$ of Zaire GP (1-308) to hTIM-1 IgV domain is -28.09 kJ/mol. Standard Gibbs energy of binding of Zaire GP (1-320) to hTIM-1 IgV domain is -26.10 kJ/mol. $\Delta_B G^o$ of Zaire GP (1-501) to hTIM-1 IgV domain is -32.67 kJ/mol. $\Delta_B G^o$ of Deglycosilated Zaire GP (1-320) to hTIM-1 IgV domain is -24.62 kJ/mol.

The dissociation constant of the Ebola virus is approximately 1000 times greater than that of SARS-CoV-2. Thus, thermodynamic limitations prevent the Ebola virus to take pandemic proportions.

Infections and epidemics are dynamic and influenced by multiple factors, including host, environmental, host genetic, nutritional, microbiome etc. Thus, it is sometimes hard to simplify infection into a purely biothermodynamic model. Biothermodynamics and bioenergetics are only one of the factors that could shed more light on the complex phenomena of infections and epidemics.

CRediT authorship contribution statement

Marko Popovic: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The author declares no conflict of interest.

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