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SEEBECK-LIKE EFFECT IN SARS-COV-2 BIO-THERMODYNAMICS

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ABSTRACT. The new coronavirus, SARS-CoV-2, relies on a pH decrease to infect the target cell and replicate its RNA. This leads to a change in the electric potential of the cell's membrane which in turn alters cell functions. Therapeutic intervention should therefore assist these cells in maintaining their natural electric membrane potential so that they can manage normal fluxes of heat and ions which are essential for survival. Results from our thermodynamic approach suggest to employ anti-SARS-CoV-2 therapeutic strategies that are capable to vary the Gibbs function, related to pH-dependent viral glycoproteins. Our approach lends theoretical credence to the potential benefit of using as starting points drugs such as chloroquine and hydroxychloroquine, while not minimizing their controversial risk profile as described recently in several COVID-19 clinical studies.

1. Introduction

Coronaviruses are a family of enveloped, spherical or pleiomorphic (80 – 120 nm) viruses, known since the 1930s (Hudson and Beaudette 1932; Belouzard *et al.* 2012). They became more wildly known in the 2002 epidemic, when a great number of people contracted SARS, *i.e.*, the severe acute respiratory syndrome. With SARS, experts pointed out coronaviruses' capabilities to jump across species (Belouzard *et al.* 2012). By the end of 2019, the SARS-CoV-2 outbreak began in Wuhan (Hubei, China) and caused the sixth international health emergency. While the complexity of the disease caused by the novel coronavirus, *i.e.*, COVID-19, shows an ever widening range of clinical manifestations including brain and kidney involvement and blood clotting abnormalities, a main focus in the fight against SARS-Cov-2 remains on its respiratory symptoms such as fever, dry cough, and even dyspnea, *i.e.*, very similar to SARS, and the 2012 Middle East Respiratory Syndrome (MERS), all spread through droplet and contact transmission (Gu *et al.* 2020). To develop effective anti-coronavirus therapeutics and vaccines, the molecular mechanism that underlies viral infection must first be better defined (Xia *et al.* 2020a). This paper is an attempt to approach this topic from a thermodynamic perspective.

The virion envelope contains some proteins, and in particular a spike protein, which plays a fundamental role as mediator for viral entry as well as for membrane fusion, which determines conformational changes of the spike protein itself. As such, infection starts by binding the viral receptor to some specific proteins on the cell membrane, in order to deliver

its nucleocapsid into the cell (Enjuanes *et al.* 2006; Perlman and Netland 2009; Belouzard *et al.* 2012). SARS-CoV fusion is supposed to be triggered by proteolytic processing of the spike protein (Matsuyama *et al.* 2005), and it enters by relying on endosomal proteases (Simmons *et al.* 2005).

In this paper, we focus our thermodynamic analysis on the pH-dependent fusion mediated by the SARS-CoV S protein in the hope to contribute to the body of knowledge that eventually will lead to more effective anti-coronavirus treatment modalities and vaccines, in accordance with current approaches (see, *e.g.*, Kandimalla *et al.* 2020).

2. Material and methods

Proteins play a fundamental role in ion transport. An ion actively crosses the membrane against its electrochemical potential whereby the necessary energy is derived either from the hydrolysis of ATP, or from the movement of a co-transported or coupled ion along its electrochemical gradient. In this context, the role played by the H^+-ATPase is fundamental, because it moves positive charges into the cell while it generates large membrane voltage (inside negative and outside positive) and a pH gradient (Stevens and Forgac 1997; Tuszynski and Kurzynski 2003; Nakanishi-Matsui *et al.* 2010). We can therefore evaluate its membrane potential by using a modified Goldman–Hodgkin–Katz equation (Goldman 1943):

$$\Delta\phi = \frac{RT}{F}\ln\left(\frac{P_{Na^+}[Na^+]_{outside} + P_{K^+}[K^+]_{outside} + P_{Cl^-}[Cl^-]_{outside}}{P_{Na^+}[Na^+]_{inside} + P_{K^+}[K^]_{inside} + P_{Cl^-}[Cl^-]_{inside}}\right) \tag{1}$$

where [A] is the concentration of the ion A, $R = 8.314$ J mol^{-1}K^{-1} is the universal constant of ideal gasses, T is the absolute temperature, F is the Faraday constant, and P is the relative permeability such that $P_{Na^+} = 0.04$, $P_{K^+} = 1$ and $P_{Cl^-} = 0.45$. The membrane potential can be related to the Gibbs energy variation and the pH variation given by Grabe *et al.* (2000), Lucia *et al.* (2014), Lucia (2015a,b), Lucia *et al.* (2015, 2016a,b,c), Lucia and Grisolia (2017), Lucia *et al.* (2017), Lucia and Deisboeck (2018), Lucia and Grisolia (2018a,b), and Lucia *et al.* (2018):

$$\Delta\phi = \Delta G + 2.3\frac{RT}{F}\Delta(\mathrm{pH}) \tag{2}$$

where G is the Gibbs energy, F is the Faradays constant, and $2.3\Delta(\mathrm{pH})$ is the physiological concentration gradient.

Protein phosphorylation is an important cellular regulatory mechanism because many enzymes and receptors (Strong 2002; Rudolph *et al.* 2006) are activated or deactivated by phosphorylation, by involving kinases and phosphatases. Kinases are responsible for cellular transduction signalling (Ardito *et al.* 2017). The phosphorylation potential, $\Delta\bar{g}_p$ [kJ mol^{-1}] can be obtained by the following equation (Tuszynski and Kurzynski 2003):

$$\Delta\bar{g}_p = -nF\Delta\phi \tag{3}$$

where n is the number of moles of ions per ATP synthesized, $F = 96.485 \times 10^3$ A s mol^{-1} is the Faraday constant, and $\Delta\phi$ is the membrane potential. In order to develop the non-equilibrium thermodynamic analysis, we must introduce the general phenomenological relations (Callen 1960; Yourgrau *et al.* 1982; Lucia and Grisolia 2020a,c,d):

$$\begin{cases} \mathbf{J}_e = -L_{11}\dfrac{\nabla\phi}{T} - L_{12}\dfrac{\nabla T}{T^2} \\ \\ \mathbf{J}_Q = -L_{21}\dfrac{\nabla\phi}{T} - L_{22}\dfrac{\nabla T}{T^2} \\ \\ L_{12} = L_{21} \qquad \text{(Onsager reciprocal relations)} \end{cases} \tag{4}$$

where $\mathbf{J}_e$ is the current density [A m^{-2}], $\mathbf{J}_Q$ denotes the heat fluxes [W m^{-2}], T depicts the temperature and L_{ij} are the phenomenological coefficients. Considering that at stationary state the net ion fluxes is null, in order to maintain the membrane potential and the pH constant, it follows that $\mathbf{J}_e = \mathbf{0}$, such that the previous equations holds to:

$$\begin{cases} \dfrac{\nabla T}{T} = -\dfrac{L_{11}}{L_{12}}\nabla\phi \\ \\ \mathbf{J}_Q = \left(L_{22}\dfrac{L_{11}}{L_{12}} - L_{12}\right)\dfrac{\nabla\phi}{T} \end{cases} \tag{5}$$

Now, we consider that:

$$\dot{Q} = \int_A \mathbf{J}_Q \cdot \hat{\mathbf{n}}\, dA \tag{6}$$

where A is the area of the membrane external surface. So, it is possible to write:

$$\delta\dot{Q} = \left(L_{22}\frac{L_{11}}{L_{12}} - L_{12}\right)\frac{\nabla\phi}{T} \cdot \hat{\mathbf{n}}\, dA \tag{7}$$

which represents a link between the heat power generated and the membrane potential gradient.

3. Results

SARS-CoV-2 infection relies on a decrease in pH in order for the virus to penetrate the cell and replicate its RNA (Belouzard *et al.* 2012). This pH variation induces a change in the membrane's electric potential, which in turn changes the normal functions of the target cell, such as in affected lung tissue. Now, we consider Eq. (2) and Eq. (7). We assume a cell to be a sphere of radius R. In this approximation, the membrane potential can be written as:

$$d\phi = \frac{T}{8\pi R\left(L_{22}\frac{L_{11}}{L_{12}} - L_{12}\right)}\delta\dot{Q} = dG + 2.3\, d\left(\frac{RT\Delta(\mathrm{pH})}{F}\right) \tag{8}$$

which relates:

- the temperature variation (inflammation) with the variation of the membrane potential;
- the activation of the inflammatory system's response to the local variation of the thermodynamic quantities, *i.e.*, the increase of the non-equilibrium conditions;
- the pH variation with the thermal power generation which induced heat fluxes;

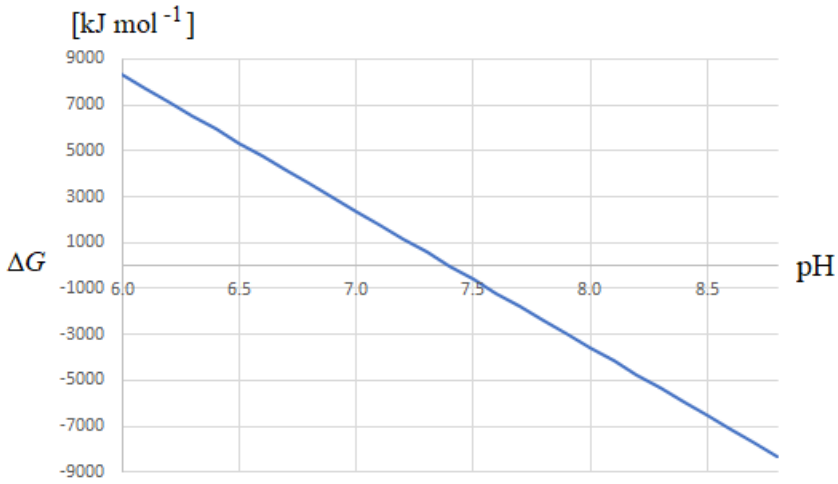

FIGURE 1. Depicted is the inverse relationship of the Gibbs G free energy variation required to maintain the membrane electric potential vs. the pH level. Considering that at physiological conditions (*i.e.*, pH = 7.4 and $\phi = -70$ mV for a human lung cell) G is around 51 kJ mol^{-1}, it follows that, at an acidic pH of 6, maintenance of ϕ at -70 mV requires that $G = 51 + 8.2 = 59.2$ kJ mol^{-1}; conversely, to maintain $\phi = -70$ mV at a pH of 8 necessitates $G = 51 + (-8.2) = 42.8$ kJ mol^{-1}.

- the spike action as a source of a Seebeck-like effect (Goupil *et al.* 2011) within the cell. Indeed, in relation to Eq. (7), any difference of temperature generates a heat flux, and as a consequence, also a membrane electric potential variation. So, in relation to Eq. (2), this induced membrane electric variation causes a variation of pH, related to the behaviour of the SARS-CoV-2 disease.

As such, lung cells must attempt to restore the conditions of stability to ensure preservation of cellular functions and proper interaction with the vascular system. As such, therapeutically, we must induce chemical (or physical) reactions capable to counter the action of the pH variation caused by SARS-CoV-2. From the previous equations, we can obtain:

$$d\Delta\phi = 0 \Rightarrow d\Delta G = -2.3\,\frac{R}{F}\,\Delta(\mathrm{pH})dT - 2.3\,\frac{RT}{F}\,d\Delta(\mathrm{pH}) \tag{9}$$

where $\Delta\phi$ is expressed by the relation (1). The Gibbs free energy variation required to maintain the membrane electric potential at the physiological level is represented in Fig. 1. It has been evaluated by using the data summarised by Pancrazio *et al.* (1989) and Hobi *et al.* (2014). We note that Eq. (2) is a general relationship for any cell membrane and in order to survive human cells must maintain their electric potential (Lucia and Grisolia 2020b). When some external or internal factor changes their pH, according to Eq. (4), cells can react in three ways:

- by changing $\Delta\phi$, but this is ineffective because the cell must also change the cell cycle;
- by changing ΔG, which means that the cell is able to continue its cell cycle, but must induce ion fluxes; this is possible if external or internal ions are available at quantities that can equilibrate the pH variation;
- by changing $\Delta\phi$ and ΔG, but it again is rather ineffective because the cell must partially change the living cell cycle.

4. Discussion and conclusions

In this paper we have developed a thermodynamic approach to shed light on a pH-homeostasis driven rationale to potentially counter infection through SARS-CoV-2. A human cell's main pH regulatory mechanism is mediated through H^+-fluxes. An accumulation of H^+ within the cell generates a negative membrane potential. To overcome the acidification, cells have developed many methods to remove the H^+, such as:

- short-term homeostasis which consumes H^+ in metabolic reactions and causes a transfer of acids from the cytosol into organelles (Roos and Boron 1981);
- membrane ion transport which includes Na^+/H^+ antiport transport in acid-loaded cells or HCO_3^-/Cl^- exchange agent extrusion in alkaline-loaded cells (Tannock and Rotin 1989): Physiologically, the Na^+-influx is preferred to H^+-influx, in order to allow the cytoplasm to be alkaline: Na^+/H^+ antiport is maintained by the Na^+/K^+ ATPase;
- H^+-ATPase pumps in specialized epithelia and in the lactate:proton symport (Anwer and Nolan 1988);
- the free Ca^{2+} ions can be maintained by two different plasma membrane transport mechanisms:
  - the Na^+-Ca^{2+} exchange, driven by the sodium gradient;
  - the Na^+-independent Ca^{2+} pump, driven by ATP: the Ca^{2+}-ATPase exchanges internal Ca^{2+} for external H^+.

It is well known that Ca^{2+} outflow is inhibited by elevated external pH (Schwiening *et al.* 1993): an increase in surface Ca^{2+} and a decrease in surface H^+ increases the recovery of intracellular Ca^{2+}. Consequently, Ca^{2+}-H^+ exchange is related to extracellular pH changes (Schwiening *et al.* 1993).

Our results suggest employing anti-SARS-CoV-2 therapeutic drugs that are capable of varying the Gibbs function as described in Eq. (9), related to pH-dependent viral glycoproteins. Since relation (9) is the Gibbs free energy required to counteract any such pH variation in an effort to maintain the cell's electric membrane potential, we must search for a therapeutic agent capable to induce this Gibbs free energy. Figure 1 indicates that in moving an infected cell from an acidic state (pH=6) back to a physiological pH value of 7.4, any such therapeutic modality would act along an energetically preferred trajectory (59.2 kJ $mol^{-1} \rightarrow 51$ kJ mol^{-1}).

Biophysical results show that the SARS-CoV-2 S protein can bind hACE2 with 10-fold to 20-fold higher affinity than the SARS-CoV S protein and that this fact seems to contribute to the high level of infectiousness (Xia *et al.* 2020a). We note that the classic anti-malaria drug chloroquine not only changes the glycosylation of the ACE2 receptor (Liu *et al.*

2020), both chloroquine and its derivative hydroxy-chloroquine are weak bases suitable to elevate the pH of acidic intracellular organelles, with particular regards to endosomes and lysosomes, essential for membrane fusion (Mauthe *et al.* 2018). This would support the notion of a potential 2-fold mechanism of these anti-malaria drugs attenuating both virus entry (through ACE2 receptor interference) and virus/cell fusion and thus its replication (through endosomal pH increase), in other words adapting ΔG to adjust for a virus-induced change in Δ(pH) (see Eq. (9)) (Wang *et al.* 2020). These beneficial effects could explain in part the promising respiratory viral load reduction results reported in a small French, open label, single-center non-randomized trial with hydroxychloroquine (and azithromycin) (Gautret *et al.* 2020); however, these data have since been followed up by disappointing results from randomized clinical trials at the US Veterans Administration (Magagnoli *et al.* 2020) and in China. The significant risk of cardiological side effects for some patient groups, most notably ventricular arrhythmia, has been made chiefly responsible (Kamp *et al.* 2020). Interestingly, it has been reported that the bradycardia caused in some patients using hydroxychloroquine seems to be due to reduced Ca^{2+} and K^{+} ion channels (Schroeder *et al.* 2017) which, however, is also being thought of as exhibiting an anti-inflammatory effect (Ornstein and Sperber 1996) that conceptually could prove useful in COVID-patients fighting so-called cytokine storms (Singh *et al.* 2020). Clearly, more research is necessary towards other both safe and effective options, to ensure that potential clinical harm does not outweigh theoretical benefit.

In summary, from Eq. (9) it follows that, theoretically, one therapeutic strategy to prohibit the acidity-assisted SARS-Cov-2 entrance into the lung cells - and consequently, to reduce the likelihood of a severe respiratory crisis – may be to facilitate maintenance of the natural membrane electric potential in the target cells, such as in lung tissue (Tannock and Rotin 1989).

In conclusion, we present a thermodynamics-based argument that controlling the cell membrane's electric potential could represent a new, promising, non-pharmacological strategy to treat complex medical conditions.

Authors' contributions

Conceptualization, U.L. and T.S.D.; methodology, U.L., G.G. and T.S.D.; software, G.G.; validation, G.G. and U.L.; formal analysis, U.L. and T.S.D.; investigation, G.G.; resources, U.L. and G.G.; data curation, G.G.; writing–original draft preparation, U.L., G.G. and T.S.D.; writing–review and editing, U.L., G.G. and T.S.D.; visualization, U.L., G.G. and T.S.D.; supervision, T.S.D.; project administration, U.L.; funding acquisition, U.L. and G.G. All authors have read and agreed to the published version of the manuscript.

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