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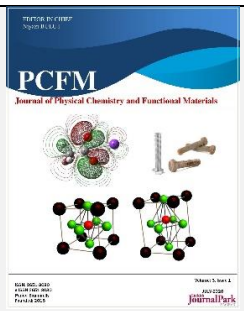

The Reactions that Increase and Decrease Co_2 Concentration During Viral Infection Like Covid-19 Virus: the Effect of Vitamin C

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ABSTRACT

It is known that some patients do not show any disease symptoms although they are sick. The human body can resist all kinds of viruses. On the other hand, the immune system may be resistant against related viruses. In the light of this thought, in viral infection treatment processes, developing an antiviral drug against the virus should be the second option. The first option is to support the immune system to block propagation medium of virus. The main purpose of this study is to determine whether the vitamin C given to the patient is sufficient to prevent the formation of low pH environment that accelerates spread of virus. For this reason, the CO_2 molecule should be easily separated from human body, that is, lungs. Viral RNA viruses indirectly might affect the carbonic anhydrase-9 enzyme, working on transmembrane, known as a pH-regulating enzyme. Interaction of vitamin C with oxidants might inhibit carbonic anhydrase-9 enzyme reactions. As a result of the interaction of vitamin C with oxidants, in this work is seen that newly formed molecular formations affect carbonic anhydrase-9 enzyme reaction and that this new molecule blocks the amino acids where zinc, which is the coenzyme of the enzyme, binds.

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1. Introduction

The Covid-19 epidemic, which was first seen in China, has showed its effect almost all over the world and every country has needed effective, fast, safe and accessible diagnostic and treatment methods. Antioxidants like vitamin C (ascorbic acid which has a strong effect potently) diminish the increase in the number of free radicals (reactive oxygen, hydroxyl ion, peroxynitrite and nitrogen species), cytokines and dysfunction caused by oxidative stress after viral infection such as Covid-19. Since it is well known that supplementation of vitamin C and E gives resistance to the body to prevent lung inflammation, uncontrolled and intense vitamin C supplements are given to patients who are affected by Covid-19 virus [1, 2]. While 100 mg of vitamin C is sufficient in a healthy person, this uncontrolled vitamin supplements in patients with Covid-19 increased up to 24 g. [3-5]. To compensate for the metabolic demand of an infected cell, a higher rate of vitamin supplements, which is capable of donating electrons, is required. This electron donating ability protects many bio molecules (proteins, lipids, carbohydrates and nucleic acids) against any external attack. During any oxidative attack, vitamin C accumulates on neutrophils at level of millimolar and tries to protect lung cells with its antioxidant feature. For this reason, vitamin C concentration is low in smokers and patients with respiratory infections. Vitamin C supplements in elderly patients has also shown to tend to heal in the patient or reduced hospital stay period [6]. In addition to vitamin C, vitamins such as vitamins D, B_{12} and nicotinamide were also used as micro nutritional supplements. Several studies mentioned that vitamin D can

act as antiviral in an immunomodulatory and anti-inflammatory way, as well as the protective effect of vitamin D [7-9]. Kandeel et al. tried to complete a knowledge gap about Covid-19 by comparing SARS and MERS CoVs virus epidemics. To do this they used molecular modelling, virtual screening and other computational techniques. They showed that the percentage of similarity of SARS is more effective when compared the other. Therefore, they compared the 20 most common used drugs, as well as ribavirin antiviral, which is used in the treatment of SARS. In addition, telbivudine (anti-hepatitis B virus) and vitamins (B_{12} and nicotinamide) were used. Combination of these drugs mentioned above was used for Covid-19 treatment. These molecular structures have been shown to increase hydrogen bond interaction by up to 10 times. For this reason, it is among the top 10 drugs that give a good docking score [10]. Remdesivir, chloroquine and favipiravir drugs were used before for SARS-CoV. Chloroquine and favipiravir, which have been used effectively both in laboratory conditions and clinically on 2019-nCoV, have been used in viral infections for many years [11]. In addition, this antiviral drugs having low toxicity effect interact with the glycosylation of cellular receptors and is known to block virus infection by increasing endosomal pH to prevent connection between the virus and the cell [11, 12].

The purpose of this study is firstly to consider the chemical reactions that occur in the lungs. Increased CO_2 density accelerates virus spread in the lung. Because this case causes the decreasing of the pH in the environment. The first aim of the study is to examine the structure of oxidant reactions that increase this density of CO_2. Another aim of this study is to determine the reaction that plays a role in the excretion of CO_2 from the body and to look at the effects of vitamin C given as uncontrolled on the lung.

2. Material and Method

O_2^- superoxide molecule reacts with NO molecule in mitochondria at diffusion rate ($0.5\text{-}1.9\times10^{10}$ $M^{-1}s^{-1}$) to form the $ONOO^-$ (peroxynitrite) molecule, which is a better nucleophile molecule than ONOOH. Peroxynitrite can easily pass through the lipid areas of the cell membrane of most biological molecules. Since it is thought to contribute to toxicity, it might provide hemolysis by protonation in water solution. Reaction of peroxynitrite molecule with CO_3^-, NO_2 and NO^- stimulated complexes in body fluid medium has a very low rate [13]. However, before its reaction with these stimulated complexes, the molecule is most likely to react with CO_2, which is abundant both in and out of the cell, and is a better electrophile than HCO_3^- (about 60 times faster, 5.8×10^4 $M^{-1}s^{-1}$). Thus, $ONOOCO_2^-$ (nitrosoperoxycarbonate anion) molecule forms in solid state in extracellular environment. This molecule is a more effective type of nitration than peroxynitrite. However, its half-life is much shorter. Cell killing ability (oxidant ability) is also reduced by CO_2

$$ONOO^- + CO_2 \rightarrow ONOOCO_2^- \quad (1)$$

Then this new oxidant absorbs another CO_2 by side of CO_2^-. And it turns into $ONOOCO_2CO_2^-$ molecule and thus as a result of the reaction, it causes 2 CO_2 and NO_3^- as product [14].

$$ONOOCO_2^- + CO_2 \rightarrow ONOOCO_2CO_2^- \rightarrow 2CO_2 + NO_3^- \quad (2)$$

As can be seen, the presence of the CO_2 molecule and the increases in its amount continue as a result of Reaction 2. This is effective even in the presence of tyrosine in the environment. Peroxynitrite molecule keeps the CO_2 molecule at intracellular, causing it to accumulate in the environment [15]. $OONO^-$ tends to react depending on the amount of CO_2. As a result of the reaction of peroxynitrite with CO_2, the formation of CO_2 again shows its catalytic effect. It is known as an exothermic reaction. Energy is required from outside for CO_3^- or HCO_3^- molecular formations. If the amount of $OONO^-$ in the environment is relatively more than CO_2, the amount of $OONO^-$ does not change after a certain time and the reaction is not worth examining as kinetic. When the amount of CO_2 increases, the amount of $OONO^-$ oxidant decreases. But the reaction takes longer. This situation is very dangerous. While CO_2 should be self-diffusing, it causes increased density of CO_2 in the environment. There are several experimental studies on this interaction. One of these studies, when the initial amount of CO_2 at 55 micro molar level interacts with the $OONO^-$ which is 0.4 mM, the amount of $OONO^-$ decreases to 0.14 mM level after 5 seconds. As the amount of CO_2 increases, the reaction time takes longer. It is a reaction that increases the CO_2 concentration in the lung [16]. In another experimental study with large amounts of CO_2, a certain amount of $NaHCO_3$ saturated CO_2 buffer was diluted (step by step from 10 mM to 0.5 mM) and peroxynitrite remained stable at 0.25 mM. [14].

Another CO_2 reaction is the interaction of hydroxyl ion, which is the product of oxidative stress inside or outside the cell, with CO_2. This reaction is a useful and desired reaction when compared to reactions (1) and (2). As a result of this reaction, bicarbonate molecule regulating pH is formed.

$$CO_2 + OH^- \leftarrow (CA) \rightarrow HCO_3^- \quad (3)$$

In order for this reaction to be physiologically useful, it must take energy from the outside. because it has an

effective energy barrier. Therefore, it occurs faster with the Carbonic anhydrase (CA) enzyme. This enzyme can also form carbonic acid as in Equation (4). Reaction (3) regulates the pH in the blood, while reaction (4) is necessary for the removal of CO_2 in the blood from the lung. Both reactions are useful enzyme reactions that regulate pH during viral infection.

$$H_2O+CO_2 \leftarrow(CA)\rightarrow H_2CO_3^- \quad (4)$$

The hydroxyl ion in Equation (3) binds to the coenzyme of the carbonic anhydrase enzyme, zinc and thus the active form of the enzyme forms. This active form interacts quickly with CO_2. Thus bicarbonate is formed and bicarbonate separates from the enzyme. Later, H_2O is bounded to the enzyme to have its inactive form. In the lung, HCO_3^- combines with H^+ released during hemoglobin oxygenation and gives carbonic acid in Equation (4). Later, with the help of the same enzyme found in red blood cells, carbonic acid is separated as CO_2 and H_2O, which diffuse to the alveolar interstisyum that will be excreted during breathing. In humans, the concentration of CO_2 outside the cell is 1-2 mM. HCO_3^- concentration is 7 mM in the cell and 25mM outside the cell [17, 18]. Carbonic anhydrase is a key enzyme that speeds up the conversion between carbon dioxide and bicarbonate. If the amount of CO_2 in the blood increases, the pH is acidic, and if the CO_2 decreases, it becomes alkaline. pH ratio in solution is provided by the Henderson-Hasselbalch equation below [19].

$$pH= 6.3 + \log ([HCO_3^-]/ [CO_2]) \quad (5)$$

It is clear that this enzyme can contribute to the lung breathing process when it operates regularly. This enzyme can be directly or indirectly affected by viral infections. Winum [20] claimed that modulators of the carbonic anhydrase enzyme can be used to treat viral infections. In the case of carbonic anhydrase enzyme suppression or inhibition, the CO_2 concentration in the environment will increase more. A certain CO_2 concentration in the alveoli can give information about the pressure of CO_2 and oxygenation and tissue perfusion. Since the partial pressure of the CO_2 molecule in the alveoli is 45 mmHg, and the O_2 coming to the lungs is less dense than the CO_2 to be excreted (40 mmHg) at an average human body temperature of 37 °C, CO_2 is thrown out with this pressure difference. Using the related enzyme, the CO_2 removal rate is 70% of the total removal rate [21]. To simplify reduction of the concentrations of carbon dioxide molecules in lungs during viral infection is important to stop propagation of virus.

Reaction 1 and 2 mentioned in this section are reactions increasing CO_2 density. The reaction that reduces CO_2 is the opposite of the reaction 4. However, for this reaction to work, Zn^{2+} ion must be connected to the carbonic anhydrase enzyme first. If this process does not occur, the leaving of CO_2 from the body occurs 10^6 times slower. In this case, virus spread continues increasingly. Stage 2 of the study is vitamin C used for therapeutic purposes. The reason why vitamin C is effective in treatment inhibits the activity of oxidants. However, there is something neglected in the literature. This is the interaction of the surrounding carbonic anhydrase enzyme with the newly formed molecular structure as a result of the struggling between vitamin C and oxidants. In this case, it is quite difficult to occur in reverse of Reaction 4. It means that the pH regulation time in the blood can take long time. With this study, the situation is shown in detail in Section 3. In order to test the interactions mentioned above, ligand and water molecules were destroyed from the carbonic anhydrase-9 enzyme with Discovery studio program [22]. H atoms were added with Autodock 1 [23]. docking with Autodock vina [24] was done. Pymol program [25] was used to display the results. Vitamin C and ascorbate mono anion used as ligand were optimized on DFT B3LYP basis set with Gaussian09 [26] program.

3. Reaction of Vitamin C with Hydroxyl Oxidant and its Effect on Carbonic Anhydrate Enzyme

The presence of vitamin C in the environment makes the interaction with the OH^- radical more effective. Since the reaction rate of vitamin C with OH^- is 10^{10} $M^{-1}s^{-1}$, the reaction of its with peroxynitrite is not so effective [27]. The reaction in Equation (3) is approximately 10^4 $M^{-1}s^{-1}$ without carbonic anhydrase enzyme. So it happens at a rather slow speed [28]. To calculate the interaction of the Covid-19 virus and the carbonic anhydrase-9 enzyme (CA-9), a quite large number of parallel processor are required. It would be interesting to try it but not shown in this study.

Vitamin-C, a powerful anti-oxidant, interacts with OH^- and reduces the presence of OH^- in the environment. So, the formation of bicarbonate caused by reaction 3 decreases. At the same time, there is an increase in amount of CO_2 in resulting from reaction 1 and so the pH begins to decrease. This creates a more effective environment for the spread of the virus [29]. Of course, this is valid when the CA-9 is inactive. If the CA-9 is actively working, reaction 3 occurs faster. OH^- molecule prefers CO_2 instead of vitamin C when considered CO_2 concentration. The interaction of Vitamin C and OH^- radical is shown in Figure 1.

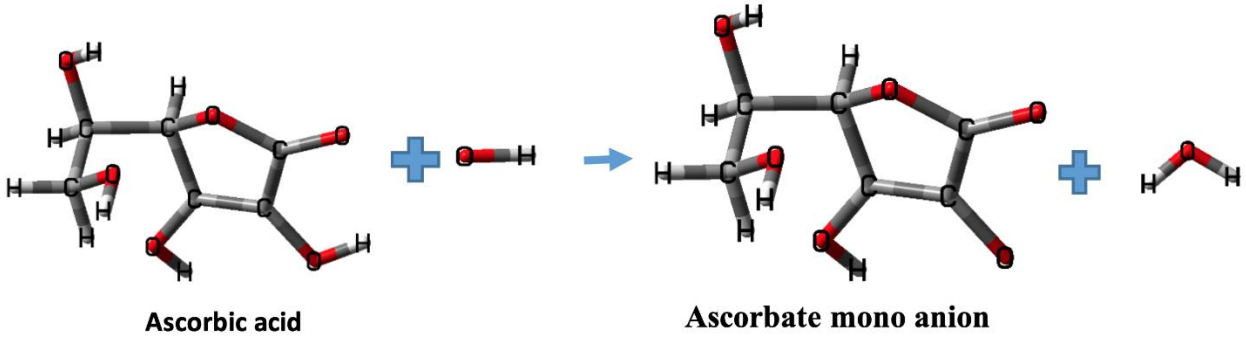

Figure1. Formation of water molecule in the interaction of vitamin C (ascorbic acid) with hydroxyl ion.

In order to examine another effect of vitamin C, it is examined whether it has an effect on CA-9. This enzyme separates the CO_2 molecule from carbonic acid and leads to diffusion from the lung. However, for this combination to occur, the zinc atom must be bound to the CA-9 enzyme and attract the OH^- ions that is present in the environment. Because Zn^{2+}, zinc ion is the coenzyme of the CA-9. Therefore, the zinc ion must first be bound to the CA-9. Without zinc, the OH^- molecule is bound to leucine and threonine amino acids with very low energy (in total of -1.7 kcal / mol) as seen in Figure 2.

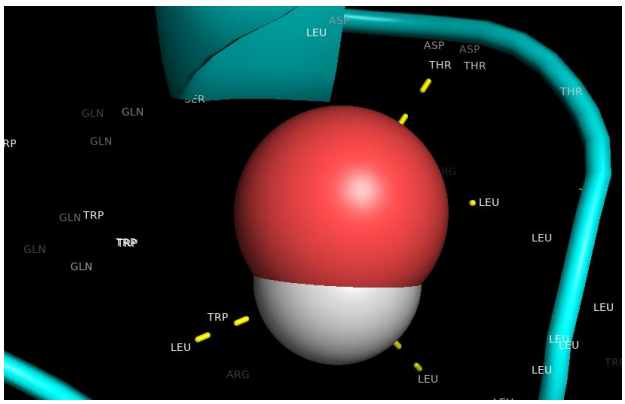

Figure 2. Effect of Free Hydroxyl Ion on Carbonic Anhydrase-9 Enzyme**.**

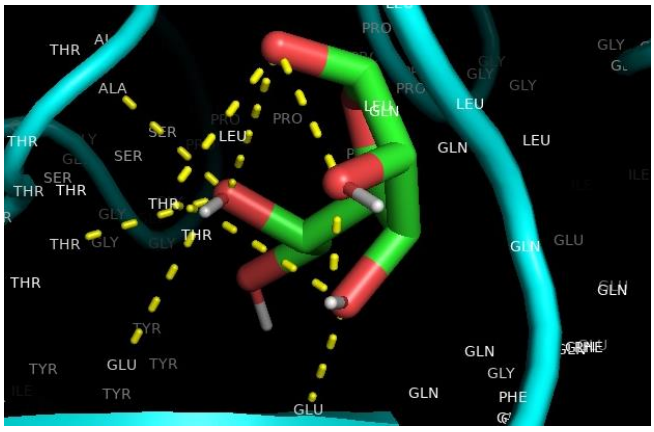

Figure3. Binding of vitamin C to several different amino acids on the enzyme.

Vitamin C doesn't bind to the CA-9 enzyme from amino acids such as histidine (His) where zinc can bind. Figure 3 shows that vitamin C binds the enzyme with -6.0 kcal / mol energy from several different amino acids within the strongest probability and least margin of error. This shows that vitamin C does not have a negative effect on the enzyme.

However, as shown in figure 1, as a result of the interaction of vitamin C with OH^- oxidant, which is free in the blood, 1 H atom is separated from vitamin C. As a result of this process, 1 H_2O molecule and 1 ascorbate mono anion are formed. This new form of vitamin C, as seen in figure 4, binds to CA-9 enzyme 3 His and 5 Thr amino acids with a total of -6.0 kcal / mol.

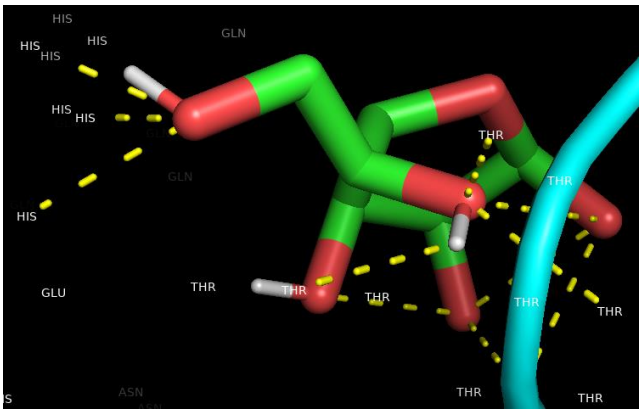

Figure 4. Binding of ascorbate mono anion to CA-9 within margin of 0%.

This connection status seriously affects the binding of zinc. Because zinc binds to the same amino acid region with the strongest probability [30]. Binding energy of zinc ion is a very low energy when compared to that of ascorbate mono anion. It must already be weakly bound because it is coenzyme. Figure 4 was calculated with 0% margin of error. Binding of ascorbate mono anion to carbonic anhydrase-9 enzyme with a margin of 2% is shown in Figure 5. As such, it is connected with a total energy of -5.7 kcal/mol. In this mode, which has slightly weaker binding energy, its connection to 1 histidine has turned into 1 Glycine (Gln).

When compared to the situation with 0% margin of error (Figure 4), the mode in Figure 5 affects the binding of zinc less. If the ascorbate mono anion molecule interacts with one more OH^- free radical, it loses its connections to histidine and tends to bind to glycine. So it moves away from region the zinc (Zn^{+2}) ion can bind. Interaction of vitamin C with oxidant for once inhibits the coenzyme of CA-9, while interacting with another oxidant of the product molecule loses this inhibiting feature. By interacting a few more times, it loses its negative feature more and more.

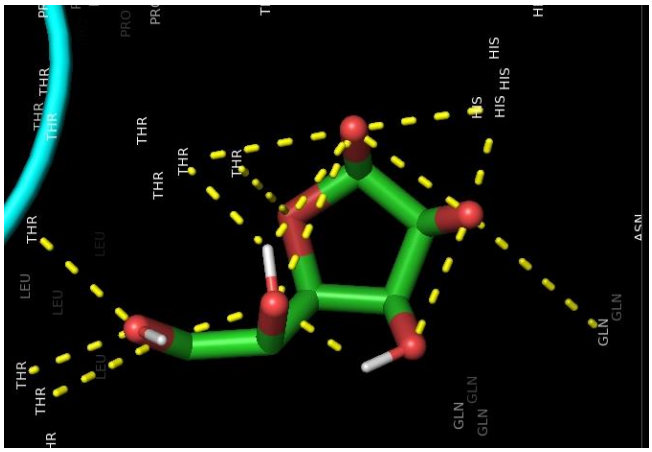

Figure5. Binding of ascorbate mono anion to CA-9 within margin of 2%.

4. Results and Discussions

In viral infections such as Covid-19, lung inflammation forms the last stage of the disease. With this stage, patients have difficulty breathing with the deterioration of the structure of the tissues of the lungs and CO_2 concentration begins to form in their lungs. As a solution they are connected to the pressurized oxygen cylinder. Besides the deterioration in the lung tissues, another factor that accumulates the CO_2 molecule in the lung is the density of peroxinitrite. The effectiveness of this oxidant can easily react with CO_2. As a result of Reactions 1 and 2, CO_2 accumulation begins to occur in the environment. In this case, the spread of virus increases. In addition to this emphasis, this study also looked at the loss of effectiveness of the CA-9 enzyme, which reduces the CO_2 concentration between cells. In such a case, ascorbate mono anion molecule, which is the product molecule in the reaction of the vitamin C given to the patient with oxidants, has been shown to inhibit this enzyme. This is a dangerous condition that accelerates the spread of viruses between cells and during the treatment process. However, this study can be supported by time-dependent with examination of different concentrations of vitamin C, oxidant and CA-9 enzyme in clinical and laboratory conditions. How could the concentrations of these molecular groups effect on the ability of Vitamin C to share H atoms and how to interpret this situation in the body environment, namely in blood plasma? How much vitamin C should be applied at which oxidant concentrations? Considering the change in the density of Hemoglobin carrying H ion, its effects on other concentrations should be considered. At the same time, the free-flowing zinc ion in the medium must be examined. Answering the above problems and questions might decrease patients stay period in the hospital.

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