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Medicinal and Analytical Chemistry International Journal
ISSN: 2639-2534

Molecular Insights into the Interaction of Vitamin C (Ascorbic Acid) with Glutathione Peroxidase: A Comprehensive Computational Study

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Research Article
Volume 8 Issue 1
Received Date: November 30, 2023
Published Date: January 31, 2024
DOI: 10.23880/macij-16000188

Abstract

This research investigates the molecular interaction between vitamin C (ascorbic acid) and glutathione peroxidase (GPX), a key enzyme in the antioxidant defense system. Oxidative stress-induced damage, caused by an imbalance in reactive oxygen species (ROS) production, is implicated in various health issues. Vitamin C, a potent antioxidant, is known for its ability to neutralize free radicals and support overall cellular health. Despite numerous studies on the protective effects of vitamin C, the molecular details of its interaction with GPX remain unclear. In this study, we employ computational methods, including molecular docking and dynamics simulations, to predict and visualize the molecular-level interaction between vitamin C and GPX. Our results reveal a favorable binding affinity, supported by negative free energy values, suggesting strong interactions. Detailed analyses of various parameters provide insights into the structural flexibility of the ligand, vibrational dynamics, and clustering characteristics. Overall, this study enhances our understanding of the molecular mechanisms underlying the beneficial effects of vitamin C in combating oxidative stress, with potential implications for therapeutic interventions.

Keywords: Molecular Interaction; Vitamin C (Ascorbic Acid); Glutathione Peroxidase (GPX); Computational Study; Antioxidant Defense System

Introduction

Antioxidants are compounds that play a crucial role in protecting cells from the damaging effects of oxidative stress [1-5]. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them. ROS, including free radicals, can cause damage to cellular structures such as proteins, lipids, and DNA, contributing to various health issues and the aging process [6]. Antioxidants work by neutralizing ROS, preventing them from causing harm to cells. They do this by donating electrons or hydrogen atoms, stabilizing the free radicals and reducing their reactivity. There are several types of antioxidants, including enzymes produced by the body and antioxidants obtained from the diet [3,4,7-9].

Vitamin C, also known as ascorbic acid ($C_6H_8O_6$), is a water-soluble vitamin that is well-known for its antioxidant properties that can donate electrons (e−) to neutralize free radicals and reactive oxygen species like singlet oxygen ($^1O_2$), like superoxide ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$) etc. By doing so, it helps prevent cellular damage caused by oxidative stress [10,11]. Vitamin C works both in the aqueous (water-soluble) and lipid (fat-soluble) phases of cells, making it effective in various cellular compartments. Vitamin C can regenerate other antioxidants, such as vitamin E. After vitamin E donates electrons (e−) to neutralize free radicals, it becomes a radical itself. Vitamin C can help regenerate vitamin E, allowing it to continue its antioxidant function. This interplay between vitamins C and E enhances the overall antioxidant defense in the body [12]. Vitamin C has the ability to chelate (bind to and remove) metal ions, such as iron and copper, which can contribute to the generation of free radicals. By chelating these ions, vitamin C helps prevent the formation of additional reactive oxygen species [13,14]. Vitamin C is essential for the synthesis of collagen, a structural protein that is crucial for the health of connective tissues, skin, blood vessels, and bones, and collagen provides structural support and helps prevent oxidative damage to tissues [15,16]. Vitamin C supports the immune system by enhancing the function of white blood cells, which play a key role in immune response. During times of infection or inflammation, the demand for vitamin C may increase, highlighting its importance in maintaining a healthy immune system [17].

Vitamin C has been the subject of numerous research studies investigating its effects on oxidative stress and various health conditions. Researchers often explore the potential benefits of vitamin C due to its well-known antioxidant properties [18]. Some studies have investigated the effects of vitamin C supplementation on exercise-induced oxidative stress. Intense physical activity can lead to increased production of free radicals, and researchers have explored whether vitamin C can help mitigate the oxidative stress associated with exercise [19]. In different research studies of arsenic induced myocardial damage also vitamin C has used as a positive control for its protective effects against oxidative damage of cardiac tissue [3,4,20,21]. Glutathione peroxidase (GPX) is an enzyme that plays a key role in the enzymatic antioxidant defense system. It is involved in the reduction of hydrogen peroxide and organic hydroperoxides, which are harmful byproducts of cellular metabolism. The level of this enzyme is reduced due to oxidative stress but it is increased in presence of vitamin C to reduce oxidative damage [3,4,21]. Though there are so many studies conducted on the activity of GPX in presence of vitamin C, but the process of molecular interaction of them is not cleared till now as per the literature study. So, the present study is designed to explore how does vitamin C is molecularly interacted with GPX.

Methods and Materials

Complement experimental approaches with computational methods such as molecular docking and molecular dynamics simulations are used to predict and visualize the interaction at the molecular level of vitamin C and GPX enzyme. For the smooth conduction of the study, we employed highly configured computer system with Autodock4, a freely available software tool for academic users, to conduct molecular docking. In performing these docking procedures, we specifically selected glutathione peroxidase enzyme as proteins from organs documented to be influenced by oxidative stress and ascorbic acid (vitamin C) as a ligand. The Figure 1 shows the name and the structure of the collected structural protein from RCSB Protein Data Bank and Figure 2 shows the ligand name and its structure obtained from PubChem. The steps involved in detailing the molecular docking processes [22] with some modifications are shown below:

Figure 1: Structure of Glutathione peroxidase (PDB ID 7FC2) in our study [23].

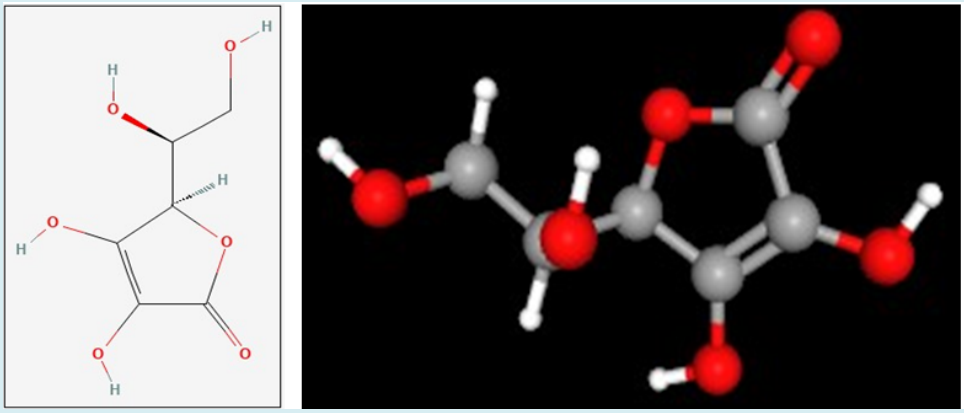

Figure 2: Structure of Ascorbic acid (Vitamin C) (PubChem CID 54670067) in our study NCBI [24].

1. The PDB and SDF files for the protein (macromolecule) and ligand were acquired from the Protein Data Bank and PubChem respectively.
2. Preparing the protein PDB files involved addressing the presence of additional waters. The files were loaded into the ADT GUI, where water molecules were identified as HOH* from a string and subsequently removed following a warning.
3. Macromolecular file preparation included the addition of polar hydrogens without bond order. Subsequently, charges were introduced using ADT, with default Kollman charges being applied (ADT automatically adds Kollman charges for a peptide).
4. In the concluding step of protein preparation, parameters were incorporated, and the files were saved as a 7fc2.pdbqt.
5. Ligand is then converted into PBD format by using OpenBable2.3.2a software.
6. Ligand, ascorbic acid is then loaded in the running ADT and parameters are prepared as per the direction and are saved as VitC.pdbqt.
7. Setting up the grid and generating the grid parameter file is crucial. To calculate docking interaction energy, a 3D box (grid) is created, enclosing the protein molecule. The grid volume must be sufficiently spacious to permit unrestricted rotation of the ligand, even in its most extended conformation. The parameters essential for constructing this grid are stored in the grid parameter file, named molecule.gpf.
8. Now a new file is created by naming 7fc2.gpf and saved in the docking folder.
9. Following this, Autogrid4 was executed to generate a map for each atom type in the ligand and produce the corresponding macromolecular file with the extension 7fc2.glg during the Run.
10. Creating the Docking Parameter File involved reading the macromolecular pdbqs and ligand.out.pdbq files. AutoDock offers various search methods, including the annealing method, the genetic algorithm, local search, and the hybrid genetic algorithm with local search. The chosen algorithm for the analysis was the Lamarckian genetic algorithm, where offspring can inherit the local search adaptations of their parents. The docking task is initiated from the 'Run' command.
11. The .dlg files are then accessed through a terminal, and the final docked energy, Gibbs free energy, and inhibition constant for each run are recorded. For the most valuable result we have determined 500 numbers of runs.

Result and Discussion

The total charge on a molecule is the sum of the charges on its constituent atoms, taking into account the number of electrons and protons. In this study we have found that the overall electric charge associated with the ligand molecule which is vitamin C is -3.001 e. A negative charge indicates an excess of electrons compared to protons, suggesting that the ligand is anionic (it has gained electrons) [25]. A rotatable bond is a single bond (sigma bond) between two non-terminal heavy atoms. Heavy atoms are those that are not hydrogen. Rotatable bonds represent the connections in a molecule that can rotate freely around their axis, allowing for different conformations or spatial arrangements of the atoms. The more rotatable bonds a molecule has, the more potential conformations it can access [26]. In Table 1, we have found that its value is 9 torsions generally indicates a moderate to high level of structural flexibility. Number of atoms in ligand is it is providing information about the molecular composition of a ligand. In chemistry, a ligand is a molecule or ion that can form a coordination complex by binding to a central metal atom. The ligand usually donates electrons to the metal, forming a coordination bond [27]. Table 1 indicates that the ligand under consideration consists of 17 atoms. These atoms could be a combination of different elements, and their arrangement and connectivity determine the structure of the ligand. Non-hydrogen atoms in ligand

provide the information about the molecular composition of a ligand, specifically indicating the number of non-hydrogen atoms in the ligand [28]. The non-hydrogen atoms in ligand of this experiment shows that it's value is 13 (Table 1). These non-hydrogen atoms could be a combination of different elements, and their arrangement and connectivity determine the structure of the ligand. Knowing the count of non-hydrogen atoms is essential for understanding the size, complexity, and potential reactivity of the ligand [28]. In Table 1, the number of vibrational degrees of freedom of ligand is 45," it indicates that the ligand molecule has a total of 45 distinct ways in which its atoms can undergo vibrational motion. This is a significant amount of vibrational flexibility and suggests that the ligand is likely to have a complex and dynamic structure [29]. Torsional degrees of freedom refer to the ability of parts of a molecule to rotate around single bonds. Each single bond connecting two non-terminal atoms introduces a torsional degree of freedom, representing a way in which the atoms on either side of the bond can rotate relative to each other [30]. In this experiment the number of torsional degrees of freedom is found 9 in Table 1 which indicates that the molecule has nine torsional or rotational degrees of freedom. So, the molecule is relatively flexible, and its structure can vary due to rotations around these specific bonds. This flexibility can have implications for the molecule's behavior in different environments or when interacting with other molecules.

Total charge on ligand	-3.001 e
Number of Rotatable Bonds in Small Molecule	9 torsions
Number of atoms in ligand	17
Number of non-hydrogen atoms in ligand	13
Number of vibrational degrees of freedom of ligand	45
Number of torsional degrees of freedom	9
Estimated Free Energy of Binding	-2.74 kcal/mol
Information entropy for clustering	0.36 (rmstol = 2.00 Angstrom)
Estimated loss of torsional free energy upon binding	+2.6847 kcal/mol

Table 1: Information of different parameters of the molecular docking.

A negative free energy of binding indicates that the binding process is energetically favorable. In other words, the binding of the ligand to the target is predicted to release energy. Gibbs Free Energy and Binding Energy are crucial parameters studied using AutoDock, as discussed earlier. The output results, represented by ΔG, provide essential insights into a closed system. AutoDock furnishes the minimum value of Gibbs free energy (ΔG) for each conformer of the ligand when docked to the protein. Additionally, it facilitates the calculation of the equilibrium binding constant (K) in each case, with these two parameters linked by the straightforward relation $\Delta G = -RT \ln K$ [31]. In Table 1 we have found that the estimated free energy of binding is -2.74 kcal/mol refers to a calculated value that represents the change in free energy associated with the binding of the vitamin C to the target protein glutathione peroxidase in a biological system suggest stronger binding interactions. Here in Figure 3 we can see the interaction of vitamin C with protein in the same way.

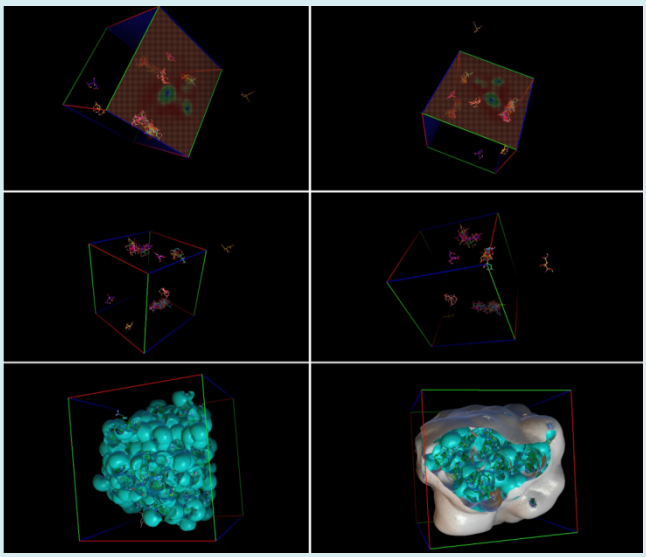

Figure 3: Interaction of ligand (Vitamin C) with protein (7FC2) [GPX6] during molecular docking.

"Entropy for clustering" likely refers to the entropy associated with the clustering of molecular conformations generated during the docking process. Molecular docking is a computational method used to predict the preferred orientation of one molecule (the ligand) when it binds to another molecule (the target or receptor) [32]. The combination of an entropy value of 0.36 and an RMSD tolerance of 2.00 Angstroms of Table 1 of this study suggests that the clustering process is yielding relatively homogeneous conformations, and the structural diversity within each cluster is limited to deviations within the specified RMSD tolerance.

A positive value indicates an increase in energy [33]. In this experiment the value of loss of torsional free energy upon binding is +2.6847 kcal/mol (Table 1) suggests that the binding process is associated with an increase in the energy required for the torsional rotation of certain bonds within

the molecules. The positive value here indicates that the binding process is associated with a higher energy cost for the torsional rotations within the molecules.

"CLUSTERING HISTOGRAM" Table 2 section describes the characteristics of a specific cluster of conformations at Cluster Rank 35 in the molecular docking simulation. The lowest binding energy Figure 4, mean binding energy, and the number of conformations in the cluster provide insights into the energetics and diversity of conformations within this particular cluster. Lower binding energies and a higher number of conformations generally indicate a more favorable and diverse set of docking poses in the cluster which is located in the Figure 5.

Cluster Rank 35	1
Lowest Binding Energy	-4.04
Run	378
Mean Binding Energy	-2.74
Num in Clus	216

Table 2: Clustering Histogram of the molecular docking.

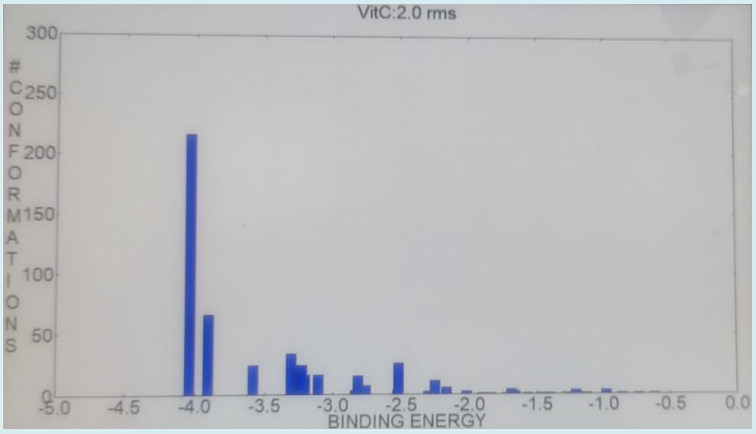

Figure 4: Binding energy of Vitamin C with GPX.

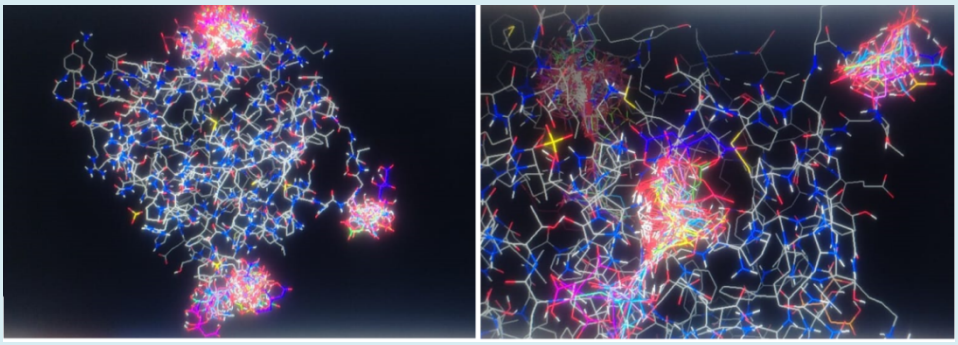

Figure 5: Favorable and diverse set of docking between vitamin C and GPX.

In summary, the presented results provide a comprehensive overview of various parameters and aspects related to the molecular docking of vitamin C with the target protein (GPX6). Here the negative charge on the ligand suggests that the ligand (vitamin C) is anionic, indicating an excess of electrons compared to protons. This information is crucial for understanding the overall charge distribution in the ligand. The presence of 9 rotatable bonds indicates a moderate to high level of structural flexibility for the ligand. This flexibility can be important for the ligand to adapt to the binding site of the target protein, allowing for different conformations. The ligand is composed of 17 atoms, providing insight into its molecular size and complexity. The arrangement and types of atoms influence the ligand's interactions with the target. The information about 13 non-hydrogen atoms gives a more specific view of the ligand's composition, excluding hydrogen atoms. This is crucial for understanding the core structure of the ligand. The high number of vibrational degrees of freedom (45) indicates a significant degree of vibrational flexibility in the ligand. This

can influence its behavior in different chemical environments. The presence of 9 torsional degrees of freedom suggests that the ligand is relatively flexible, and its structure can vary due to rotations around specific bonds. The negative value indicates that the binding of the ligand to the protein is energetically favorable. A lower value suggests a stronger binding interaction. The entropy value of 0.36, with an RMSD tolerance of 2.00 Angstroms, suggests that the clustering process has yielded relatively homogeneous conformations. This information is vital for understanding the diversity and reliability of the docking results. Estimated Loss of Torsional Free Energy upon Binding (+2.6847 kcal/mol) which is a positive value suggests an increase in the energy required for the torsional rotation of bonds upon binding. This information provides insights into the structural changes associated with the binding process. The information presented in the clustering histogram provides details about a specific cluster at rank 35. The lowest binding energy, mean binding energy, and the number of conformations in the cluster give a detailed view of the energetics and diversity within this cluster.

Conclusion

The current comprehensive computational study sheds light on the molecular interaction between vitamin C (ascorbic acid) and glutathione peroxidase (GPX), a crucial enzyme in the antioxidant defense system. The negative free energy values obtained from molecular docking signify a favorable binding affinity, indicating strong interactions between vitamin C and GPX. The detailed analyses of various parameters, including the ligand's structural flexibility, vibrational dynamics, and clustering characteristics, enhance our understanding of this interaction at the molecular level.

Vitamin C's ability to neutralize free radicals and support cellular health is well-established, and our study provides molecular insights into its interaction with GPX. The presented results offer a nuanced understanding of the structural features and dynamics associated with the binding process. Furthermore, the study highlights the potential implications of this interaction for therapeutic interventions aimed at combating oxidative stress-induced damage, a factor implicated in various health issues.

This research contributes valuable information to the existing body of knowledge regarding the molecular mechanisms underlying the protective effects of vitamin C. The findings not only advance our understanding of the antioxidant defense system but also pave the way for future studies and therapeutic developments in the realm of oxidative stress-related conditions. Overall, our computational study serves as a significant step forward in unraveling the intricate details of the molecular interplay between vitamin C and GPX.

Statement and Declaration

Conflicts of interest

Authors do not have any conflict of interest.

Funding

Not applicable.

Acknowledgements

I am thankful to all the participants and authors for their cooperation, continued support and hard work for the successful completion of this study.

Data Availability Statement

Raw data may be available upon request.

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