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BINDING EXPEDIENT OF PHENOLIC ACIDS FROM THE PLANT *GRAPTOPETALUM PARAGUAYENSE* E. WALTHER TO VIRAL DNA POLYMERASE AMINO ACIDS: A THEORETICAL INSIGHT[1]

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Abstract: *Among the most common infections are those caused by human herpes viruses, including Herpes Simplex virus type 1 and type 2 (HSV-1 and HSV-2) spread worldwide. Common therapies for herpes infections employ nucleoside analogues, such as acyclovir, and target the viral DNA polymerase, essential for viral DNA replication. Systemic application of these agents is often limited by the development of drug-resistance or toxicity, especially in immunosuppressed patients. A better understanding of the herpes virus replication will help the development of new safe and effective broad-spectrum anti-herpetic drugs that fill an unmet need. Recently we found that the total methanol extract from succulent plant Graptopetalum paraguayense E. Walther demonstrates a significant inhibitory effect on HSV-1. Since virus-encoded DNA polymerase appears to be a key feature in the replication of large DNA viruses such as HSV, we present theoretical investigations on the binding expedient of phenolic acids from this fraction to viral DNA polymerase amino acids. Phenolic acids such as gallic acid, is thought to be the key compound exhibiting antioxidant and antiviral activity. MOE 2016 software package was used to dock gallic acid structures in the active site defined in published XRD (X-ray diffraction) structures of the Herpes Simplex Virus 1 DNA Polymerase. The structure was protonated according to implemented Protonate3D algorithm and was scored according to implemented GBVI/WSA dG scoring function. According to this scoring function, gallic acid has optimal interactions with the receptor. From the results based on the molecular docking methods, we have modeled some hydrogen-bonded complexes between the phenolic and amino acids. The data received from our quantum-chemical calculations suggest that gallic acid could form stable complexes with amino acids from the DNA polymerase active site. The calculations were performed at B3LYP/6-31+G(d,p) level of theory using GAUSSIAN 09 software package.*

Keywords: *Graptopetalum paraguayense E. Walther, DNA polymerase, Herpes Simplex virus, docking, quantum-chemical calculations, hydrogen-bonding, gallic acid;*

[1] Presented a report of November 1, 2019 with the original title: Теоретично изследване върху възможността за свързване на фенолните киселини от *Graptopetalum paraguayense* E. Walther към аминокиселините на вирусна ДНК полимераза Теоретично изследване върху възможността за свързване на фенолните киселини от *Graptopetalum paraguayense* E. Walther към аминокиселините на вирусна ДНК полимераза

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INTRODUCTION

Herpes viruses are large DNA viruses. There are around 100 types of Herpes viruses but only 8 typically infect humans and have clinical significance (Weller and Coen, 2012). When replicating they need DNA polymerase enzyme which is carried by the virions to accurately replicate their genomes. As a key component of the replication, viral DNA polymerases are the main target of the currently used antiviral therapies (Coen and Schaffer, 2003). Most of the antiherpetic drugs are nucleoside analogues which are a rich source of antiviral agents. Systemic application of these agents is often limited by the development of drug-resistance or toxicity, especially in immunosuppressed patients which highlights the need for new therapeutic options. Plant extracts and plant-derived compounds represent an important source of new potential antiherpetic drugs.

Crassulaceae family had shown high virus neutralizing activity (Ürményi et al., 2016). The succulent plant *Graptopetalum paraguayense* E. Walther (GP) is a member of this family. The plant is native to Mexico and is popular in Chinese herbal medicine. It is a widely consumed plant food in Taiwan and it is used in folk medicine. This herb possesses several health benefits: anti-inflammatory (Chen et al., 2016), antioxidant (Chung et al., 2005) and antineoplastic activities (Hsu et al., 2015).

There are no studies about the antiviral effect of the plant *Graptopetalum paraguayense E Walter*. Recently we found for the first time that the total methanol extract from GP demonstrates a significant inhibitory effect on wild-type HSV-1 strain Victoria. The results from the cytotoxicity investigation of the tested extract showed a high cell tolerable concentration range (Zaharieva et al., 2019).

There are few scientific articles on the chemical composition of *Graptopetalum paraguayense*. Several glycosides of the flavonoids kaempferol and quercetin were found by Liu et al. (Liu et al., 2015). The total phenol and anthocyanin contents were analyzed by Chung et al. (Chung et al., 2005) to evaluate the anti-oxidative activities of different extracts of GP. The highest total phenol and anthocyanin contents were obtained from GE50 of GP and could be attributed to the radical-scavenging activities of the extract, probably due to gallic acid (GA). The anti-herpetic activity demonstrated in our investigation is probably also related to the presence of phenols, and in particular gallic acid.

Since virus-encoded DNA polymerase appears to be a key feature in the replication of large DNA viruses such as HSV, we present theoretical investigations on the binding expedient of gallic acid to viral DNA polymerase amino acids using quantum chemical methods.

RESULTS AND DISCUSSION

To clarify the interactions between GA and residues inside the cavity of the active site of DNA polymerase (DNA Pol) with 2GV9 pbd code (Liu et al., 2006) a docking simulation was performed. MOE 2016 software package was used to dock gallic acid structures in the active site defined in published XRD (X-ray diffraction) structures of the Herpes Simplex Virus 1 DNA Polymerase.To prepare the XRD structure used for the docking procedure, we removed the non-peptide species (water, acyclovir and ions) (Fig. 1). In the next step all possible structural conformers of gallic acid were docked inside the pocket and the interaction energy was elucidated according to GBVI/WSA scoring function (Corbeil et al., 2012). During the docking procedure α-C-skeleton of the protein was frozen while other residue functional groups were treated with induced fit algorithm. The results obtained were ranked by their interaction energy. The possible interactions between the phenolic acid and the amino acids in the DNA Pol pocket are shown on Fig. 2.

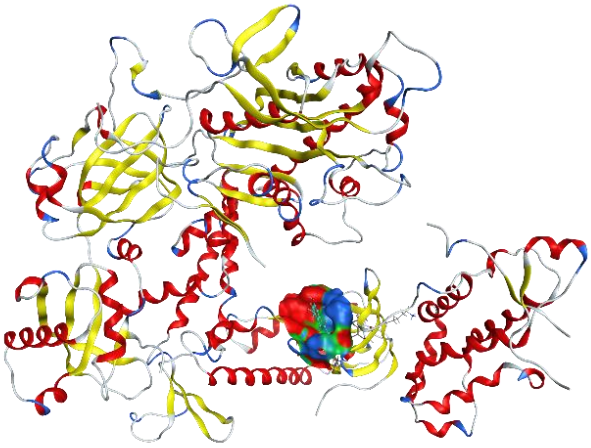

Fig. 1. Active site of DNA polymerase with 2GV9 pbd code**.**

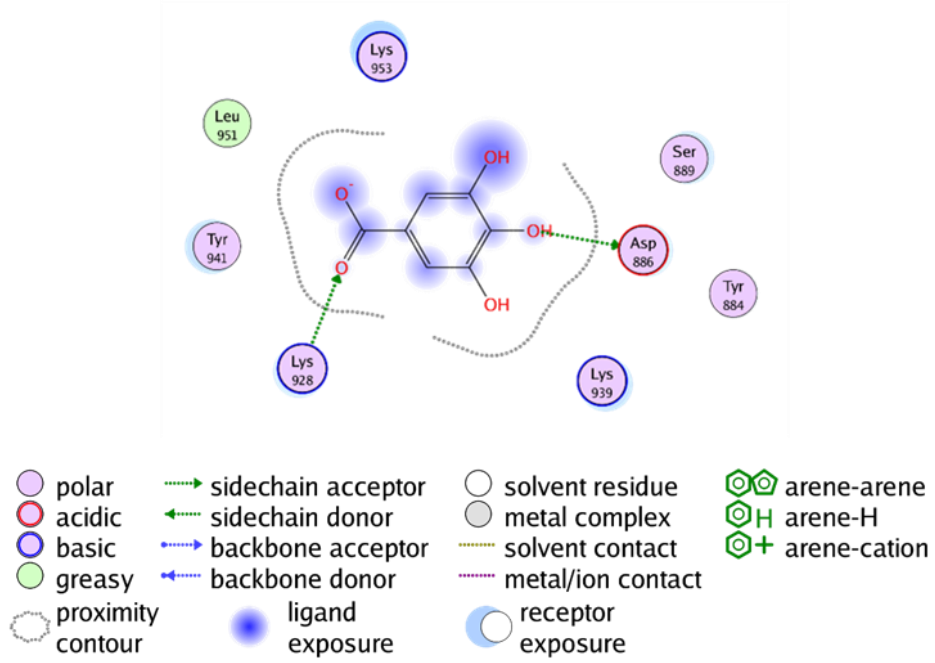

Fig. 2. Interactions of gallic acid inside the active center of DNA polymerase

To study the two binding modes between GA (by -COOH and -OH groups) and DNA Pol active site quantum-chemical calculations at B3LYP/6-31+G(d,p) (Lee et al., 1988), (Becke, 1993) theoretical level were performed by GAUSSIAN 09 suite of programs (M. Frisch, et al., 2009). On the other hand the amino acids could bind to GA by their -COOH and amino groups. To simulate the interactions between GA and amino acids, hydrogen bonded complex between the carboxylic groups of the two acids was modelled (Fig. 3). Since aspartic acid is one of the amino acids in the structure of the DNA Pol active site (Liu et al., 2006) we modelled gallic acid-aspartic acid complex. Initially, the geometries of GA, aspartic acid and their complex were optimized at B3LYP/6-31+G(d,p) level. The interaction energy (E_{int}) of the complex was estimated to be 16.94 kcal mol^{-1} by formula 1:

$$E_{int} = E_{GA} + E_{Asp} - E_{complex} \quad (1)$$

The value of E_{int} as well as the strong intermolecular hydrogen bonds in the model presented on Fig. 3 show that GA forms stable hydrogen-bonded complex with aspartic acid.

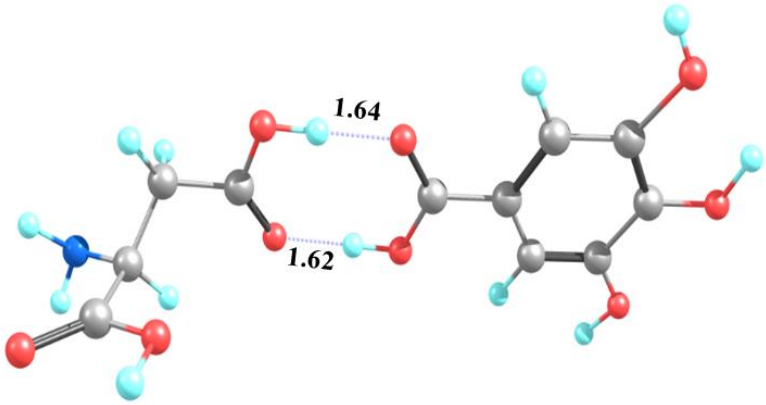

Fig. 3. Gallic acid-aspartic acid complex, optimized at B3LYP/6-31+G(d,p) level. The length of the intermolecular hydrogen bonds is in Å.

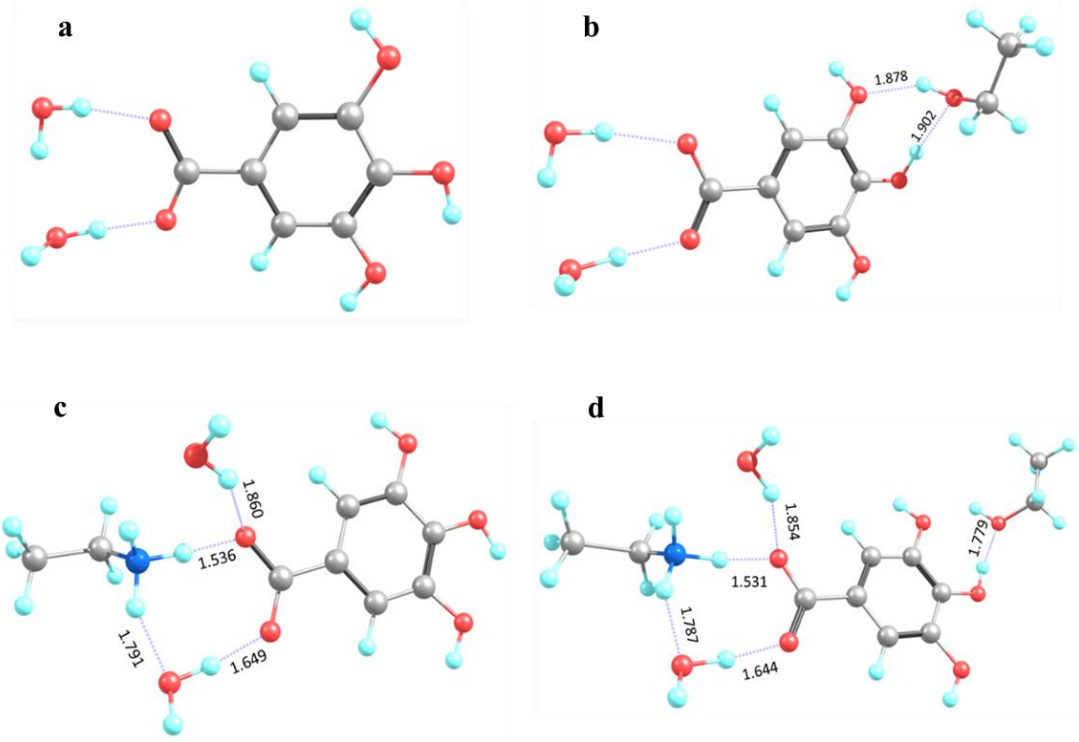

Fig. 4. Structures of the gallic acid complexes calculated at B3LYP/6-31+G(d,p) level: a) GA^- +2H_2O; b) GA^- +2H_2O+EtOH; c) GA^- +2H_2O+ $EtAH^+$; d) GA^- +2H_2O+EtOH+ $EtAH^+$. The length of the intermolecular hydrogen bonds is in Å.

To simulate the interactions between GA and DNA Pol active site four hydrogen-bonded complexes were considered (Fig. 4). In order to resemble the amino acids in the enzyme active site (Fig. 2) we mimicked them with ethanol and protonated ethylamine (EtAH+). Since in physiological conditions the phenolic acid carboxyl groups are deprotonated and the amino groups are in protonated form we made complexes of deprotonated gallic acid (GA^-) and protonated ethylamine. Because of the presence of water in the enzymes active sites, we modelled our complexes with two water molecules bounded by intermolecular hydrogen bonds to deprotonated carboxyl group of GA. These bonds stabilized the complexes of GA with EtAH+. One EtAH+ molecule was situated in the same region forming hydrogen bonds with GA and water. To estimate the influence of the hydrogen bonds forming between –OH groups of GA and DNA Pol residues the ethanol molecule was located close to the hydroxyl groups of the acid (Fig. 4b). All interactions

described above are taken into account in the complex shown on Fig. 4d. The hydrogen bonds formed between ethanol and the hydroxyl groups of the gallic acid (Fig.4b) are shortening in the presence of ethylamine (Fig.4d).

The geometry optimization of the molecular structure of complexes: $GA^- + 2H_2O$, $GA^- + 2H_2O + EtAH^+$, $GA^- + 2H_2O + EtOH$ and $GA^- + 2H_2O + EtOH + EtAH^+$ as well as GA^-, $2H_2O$, EtOH and $EtAH^+$ was performed at B3LYP/6-31+G(d,p) level (Fig. 4).

The interaction energy of the $GA^- + 2H_2O + EtOH + EtAH^+$ complex was calculated by:

$$E_{int} = (E_{water} + E_{GA^-} + E_{EtOH} + E_{EtAH^+}) - E_{complex} \quad (2)$$

The interaction energy of the $GA^- + 2H_2O + EtOH + EtAH^+$ complex was found to be 32.97 kcal mol^{-1}. The value of E_{int} as well as the strong intermolecular hydrogen bonds in the model presented on Fig. 4d show that GA forms stable hydrogen-bonded complex with DNA Pol active site residues.

CONCLUSION

For investigation on the binding expedient of the gallic acid from *Graptopetalum paraguaense* to DNA Pol participating in HSV-1 replication, molecular docking and quantum-chemical calculations were applied. The results obtained show considerable affinity of the gallic acid towards enzyme active site. The interaction energy of the complex is 32.97 kcal mol^{-1}. Therefore, we can conclude that the complex formed (Fig. 4d) is stable and gallic acid demonstrates great affinity for binding to the active site of DNA Pol where it can exhibit its inhibitory properties.

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