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Computational study of bindings of olive leaf extract (OLE) to HIV-1 fusion protein gp41

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Abstract **Recent experimental study found that OLE (olive leaf extract) has anti-HIV activity by blocking the HIV virus entry to host cells [Lee-Huang, S., Zhang, L., Huang, P.L., Chang, Y. and Huang, P.L. (2003) Anti-HIV activity of olive leaf extract (OLE) and modulation of host cell gene expression by HIV-1 infection and OLE treatment. Biochem. Biophys. Res. Commun. 307, 1029; Lee-Huang, S., Huang, P.L., Zhang, D., Lee, J.W., Bao, J., Sun, Y., Chang, Y.-Tae, Zhang, J.Z.H. and Huang, P.L. (2007) Discovery of small-molecule HIV-1 fusion and integrase inhibitors oleuropein and hydroxytyrosol. Biochem. Biophys. Res. Commun. 354, 872–878, 879–884]. As part of a joint experimental and theoretical effort, we report here computational study to help identify and characterize the binding complexes of several main compounds of OLE (olive leaf extract) to HIV-1 envelop protein gp41. A number of possible binding modes are found by docking oleuropein and its metabolites, aglycone, elenolic acid and hydroxytyrosol, onto the hydrophobic pocket on gp41. Detailed OLE-gp41 binding interactions and free energies of binding are obtained through molecular dynamics simulation and MM-PBSA calculation. Specific molecular interactions in our predicted OLE/gp41 complexes are identified and hydroxytyrosol is identified to be the main moiety for binding to gp41. This computational study complements the corresponding experimental investigation and helps establish a good starting point for further refinement of OLE-based gp41 inhibitors.**

Keywords: HIV-1 entry inhibition; Olive leaf extract (OLE); Oleuropein; Hydroxytyrosol; Binding modes; Gp41; Binding free energy; PBSA

1. Introduction

Human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein (Env) plays an important role in the early stage of HIV entry. Its surface subunit gp120 is responsible for virus binding to receptor and co-receptors, and the transmembrane subunit gp41 mediates fusion of the virus with the target cell [1–5]. The crystal structures of the six-helix bundle in gp41 show that three helices from the N-peptides (the N-helices) associate to form the central trimeric coiled-coil and three helices from C-peptide region (the C-helices) pack obliquely in an anti-parallel configuration into the highly conserved hydrophobic grooves on the surface of the central coiled-coil. In each of the grooves, there is a highly conserved hydrophobic deep cavity, formed by the cavity-forming sequence in the NHR region, which is critical for viral fusion and stability of the 6-HB (helix bundle) [6]. Gp41 is an important target for developing AIDS drugs besides RT (reverse transcriptase), protease and integrase. Inhibition of gp41 can effectively block HIV-1 entry into human cells and thus prevent new infection of AIDS virus [7–10].

Much effort has been devoted toward developing effective small molecular inhibitors of gp41 with little success. The unique 6-HB structure of gp41 makes it difficult to identify a suitable small molecule inhibitor that can bind effectively to gp41. Recent experimental investigation by Lee-Huang and co-workers has found that OLE (olive leaf extract) shows strong anti-HIV activity. Their research shows that OLE inhibits acute infection (new virus infection) and cell-to-cell transmission (virus replication) of HIV-1 [11]. One of the suspected targets for OLE action is HIV-1 gp41 which is responsible for HIV entry into normal cells. In order to establish HIV protein targets of OLE and its inhibitory action at molecular level, a joint theoretical and experimental effort has been carried out to help achieve this goal [12]. OLE is known to contain a mixture of polyphenolic compounds, among them oleuropein, oleuropein aglycone, elenolic acid and hydroxytyrosol (see Fig. 1), which are readily absorbed and bioavailable. The biological activities of OLE are mainly derived from these compounds [13,14]. In this work, we report computational study to help identify and characterize specific actions of the main ingredients of OLE and their binding interactions with gp41. To complement the experimental investigation, we performed systematic computational studies to investigate possible binding complexes of OLE/gp41 through molecular docking, MD (molecular dynamics) simulation and free energy calculations. Specific binding modes from docking studies are analyzed and MD simulation is performed to study molecular interaction, binding mechanism, stability of the binding complexes and binding affinities for oleuropein and several of its main metabolites with gp41.

2. Computational methods

Each ligand was first optimized at the AM1 level using the Gaussian 98 package [15] and the minimized structure was used to calculate the HF/6-31G* electrostatic potential (ESP). The atomic charges used for

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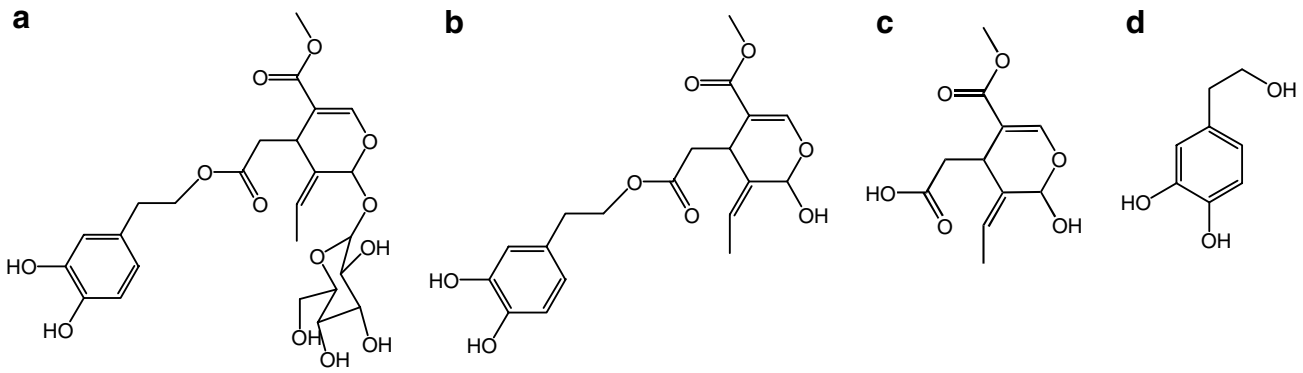

Fig. 1. Compounds in OLE mixture: oleuropein (a), oleuropein aglycone (b), elenolic acid (c) and hydroxytyrosol (d).

molecular mechanics calculations were derived from the ESP by using the RESP [16] program implemented in the AMBER 8.0 [17] package. For the ligands we study here, the binding orientations were first estimated using AutoDock 3.0.5 [18]. There is a highly conserved large hydrophobic cavity located at the N-terminal of N-36 coiled-coil core structure, including conserved residues such as Leu565, Leu566, Thr569, Val570, Trp571, Gly572, Ile573, Lys574, Leu576, and Gln577 [6], occupying this cavity by small molecules will interrupt the formation of six-helix fusion structure, thus inhibit the HIV membrane fusion process. The preliminary docking studies show that the hydrophobic cavity located at the N-terminal of the N36 trimer is a relatively favorable site for the docking of small molecule. We thus used this hydrophobic cavity to generate the receptor site and the energetic grids for the docking calculations. To obtain all the possible binding orientations, flexible docking was performed in which single bonds outside the rings were set free to rotate. During the docking process, conformational search was performed using the Solis and Wets local search method, and the Lamarckian genetic algorithm was applied to find minimum energy structure of the ligand–receptor complexes. The docking structures obtained were then minimized in the fixed protein using 200 steps of Steepest Descent followed by 2000 steps of Conjugate Gradient.

Molecular dynamics simulations were carried out using the SANDER module of the AMBER8.0 program [17] with the ff03 version of Amber force field [19]. For each gp41-ligand binding mode obtained from AutoDock, about 10000 TIP3P water molecules with 10 Å buffer were added around the complex. Eleven K^+ counterions were added to maintain the neutrality of the system. The simulations were carried out at 300 K with a time step of 2.0 fs. The non-bonded cutoff was set to 10.0 Å and SHAKE algorithm was applied for all the bonds involving hydrogen atoms. After minimization of 1500 steps and equilibration for 150 ps, complex conformations were collected every 1 ps for the following 3 ns simulation. Finally, 100 snapshots were collected from the region with stable fluctuation for post-processing analysis and for free energy calculations.

For each snapshot collected during the MD simulation, both ligand–protein interaction energies (ΔE^{vdw}, ΔE^{elec}) and the electrostatic contribution (ΔG^{PB}) to the solvation energy were calculated with the PBSA program of AMBER 8.0 [17]. Here, the single trajectory approach is applied to estimate the energies. This approach means that the thermodynamic data are extracted from a single trajectory of the protein–ligand complex based on the assumption that the bound protein and bound ligand conformations are similar to their free conformations. Estimation of energies in this manner has proven successful in many studies. Part of the reason for the success of this approach is the cancellation of errors that hides the effect of incomplete sampling. A logically better approach, limited in practice due to larger fluctuations and errors, is the use of separate trajectories where the energies are estimated from three separate MD trajectories of protein–ligand complex, free protein, and free ligand. Due to sampling limitations, the separate trajectory approach appeared to be significantly less stable in numerical study. The non-polar part of the solvation energy (ΔG^{SA}) is estimated using the simple empirical relation: $\Delta G^{SA} = \gamma A + b$, where A is the solvent-accessible surface area that is estimated using Sanner's algorithm implemented in the MSMS program [20] with a solvent probe radius of 1.4 Å and the PARSE atomic radii parameters [21]. γ and b are empirical constants and are set to 0.00542 and 0.92 kcal/mol, respectively [21]. The energy terms obtained with MM-PBSA approach [22] were then averaged over 100 time frames. The normal mode calculation to estimate the entropy contribution is somewhat problematic and time-consuming [23,24]. One normal mode calculation was performed by using Nmode module in the AMBER 8.0 [17] for each ligand and only residues at the nearest two N36 helices were included in the entropy calculations.

3. Results and discussion

Studies were carried out to dock, respectively, oleuropein and its three metabolites to the hydrophobic pocket of gp41. In general, molecular docking give quite different lowest energy orientations, especially for a molecule with many degrees-of-freedom (DOF) and the weak binding. Therefore, in order not to miss the energetically favorable orientations, our docking result is analyzed by cluster analysis and several different binding modes with the best scores and the largest populations for each molecule were selected for further MD study and analysis. Three nanosecond molecular dynamics simulations were performed for all the binding modes of oleuropein, oleuropein aglycone, elenolic acid and hydroxytyrosol. Although for most of the modes the systems were well equilibrated after 400 ps MD simulations, the 100 snapshots (every fourth snapshot of the 400 collected snapshots) for further processing analysis were selected after equilibration with the lowest RMSD fluctuations. The binding structure for the mode with the lowest free energy for each ligand was further analyzed.

All the binding energies and entropies from the normal mode analysis have been calculated and listed in Table 1. The binding mode that has the lowest binding free energy is supposed to be the most favorable binding mode. Our result shows that most binding modes with the lowest free energies have large negative electrostatic interaction energies, implying that the electrostatic interaction plays a dominant role in stabilizing the ligand–gp41 complex. We notice, however, that elenolic acid is the only exception with relatively less attractive electrostatic interaction as shown in Table 1. As a result, we could not find any elenolic acid–gp41 complexes with negative binding free energies.

Considering various binding modes for each ligand, complexes of gp41 with oleuropein and oleuropein aglycone (oleuropein non-sugar compound) have relatively larger van der