Recognition of Resveratrol by the Human Estrogen Receptor-Alpha: A Molecular Modeling Approach to Understand Its Biological Actions

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Key Words

Human estrogen receptor- α · Resveratrol · Phytoestrogens · Molecular modeling

Abstract

Objectives: Resveratrol (RSVL) is an edible phytoestrogen with multifaceted health benefits that may originate from binding to the estrogen receptors. Despite its structural similarity to the estrogen receptor- α (ER α) agonist diethylstilbestrol (DES), RSVL showed distinct biological profiles in estrogen-responsive biological systems. The molecular basis of such biological profiles has been undefined. **Methods:** We considered possible orientations for RSVL in ER α binding pocket. These conformations have been analyzed based on: (i) alignment with the key pharmacophoric elements of DES; (ii) computational energy of interaction, and (iii) pattern of accommodation at the ER α binding pocket. The characteristics of the most favored RSVL orientation have been compared with those of DES. **Results:** Both RSVL and DES interacted with the catalytic amino acid triad of the ER α pocket (His524, Arg394 and Glu 353). However, unlike the ER α agonists DES and estradiol (E₂), RSVL formed three additional hydrogen bonds with Gly521 and Leu525, two paramount ligand recognition residues, and with Met343 at the ER α binding cavity. Lastly, RSVL displayed a more favorable energy of interaction with the ER α binding cavity.

Conclusions: The present study suggests, for the first time, that RSVL is well recognized by the human ER α but in a manner distinct from the pure agonists DES and E₂. These variations may well entail the unique biological responses of RSVL in ER-responsive systems.

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Introduction

Resveratrol (*trans*-3,4',5-trihydroxystilbene, RSVL; fig. 1) is a naturally occurring polyphenolic stilbene that is commonly found in grapes and berries [1]. Its availability in red, but not white wine suggests that RSVL protects against cardiovascular diseases in certain populations, such as the French and Greek, despite having a diet that is rich in fat [2]. This dogma, commonly designated as 'the French paradox of red wine', has triggered an explosion of research in the last decade, which revealed that RSVL confers antimitotic/antiatherotic, antineoplastic, antioxidant, antiplatelet, and anti-inflammatory actions [3–7]. A great deal of these biological profiles was attributed to interaction with the estrogen receptor machinery [8]. Not surprisingly, therefore, RSVL was classified as a phytoestrogen. The phytoestrogen family is a group of nonsteroidal dietary products which can modulate the function of estrogen-dependent biological systems [9]. Although the biological actions of phytoestrogens are well established in

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Fig. 1. Energy-minimized structure of RSVL and the crystallographic structure of DES and E₂.

Fig. 2. a Flat-ribbon presentation of the crystallographic structure of DES-ER α -LBD showing DES (space-filling) and the catalytic triad residues (His524, Arg394 and Glu353, dark structures) at the ER binding domain. b Crystal structure of DES at the ER α binding pocket showing H-bonding interactions of DES with the catalytic triad residues (dashed lines). Numbers indicate the length of HB in ångström.

humans and animals, their exact mechanism of action remains unclear. Intriguingly, RSVL bears the closest structural features to the estrogen receptor- α (ER α) agonist diethylstilbestrol (DES, fig. 1). However, it displayed a mixed agonist/antagonist activity on the ER α [10]. The underpinnings of these responses are barely understood. Currently, a molecular modeling study was conducted to help clarify these ambiguities. The molecular modeling profiles of RSVL have been delineated and further compared with those of DES and the prototype estradiol (E₂, fig. 1). This study is the first to highlight the mode of recognition of RSVL by the human ER α .

Computational Methods

Receptor Structure

Starting coordinates of the human ER α ligand-binding domain in complex with DES (DES-ER α -LBD, code ID 3ERD) was obtained from the Protein Data Bank of Brookhaven National Laboratory (fig. 2) [11].

Molecular Structure of RSVL

The starting conformation for *trans*-RSVL used for receptor docking studies was generated from random conformational search performed using the AMBER force field as implemented in GRO-MOS [12]. The resulting lowest-energy conformer was then used for docking studies. Charge calculations were determined using the semiempirical quantum mechanics method 'AM1' as implemented in HyperChem 6 [13]. The resulting lowest-energy conformer of RSVL was prepositioned on DES at the ER α binding domain (fig. 2) using a least-squares multifitting of the selected atoms within the DES ligand (pharmacophoric elements: A, B rings and the stilbene bond) [14]. Once prepositioned, DES was deleted and RSVL was optimally docked in the ER α binding pocket in the possible orientations (as detailed in the text) using the Flexidock routine implemented in PowerFit [15] (fig. 3). Both hydrogen bond donors and acceptors within the pocket surrounding the ligand (Glu353, Arg394 and His524), the ligand itself and the torsional bonds were defined. The best docked receptor-ligand complex from Flexidock then underwent a three-step minimization: first, the nonring torsional bonds of the ligand were minimized in the context of the receptor, followed by minimization of the side chain residues within 8 Å of the ligand while holding the backbone and residues Glu353 and Arg394 fixed. Finally, energy minimization followed by geometric optimization of both the ligand and receptor was conducted until an energy convergence of

Fig. 3. All possible orientations of the lowest energy-minimized RSVL. The structures were generated by rotation around the stilbenic double bond and/or exchange of the phenolic rings.

0.05 kcal/molÅ was reached [16, 17]. Energy of interaction was calculated for each RSVL-ERα model after minimization as the difference between the energy of the complex and individual energies of the receptor and the ligand (RSVL) [18, 19]:

$$E_{\text{interaction}} = E_{\text{Complex}} - (E_{\text{Ligand}} + E_{\text{Receptor}}).$$

Results and Discussion

RSVL is an edible stilbene of plant origin with a myriad of beneficial biological effects [1–3]. An elegant population study demonstrated that dietary intake of RSVL can attain plasma levels that are evidently biologically active [20]. Owing to striking structural similarity to the ER agonist DES, RSVL has been established as a phytoestrogen [2]. Subsequent studies for RSVL revealed distinct ligand characteristics that combined both agonistic and/or antagonistic effects on the estrogen machinery. In a detailed study that employed extensive biological testing protocols, two structurally unrelated phytoestrogens, RSVL and naringen (a flavanoid), were comparatively investigated [21]. Both ligands exhibited agonistic activity in the absence of E₂. However, only RSVL displayed antagonistic activity when present with E₂. Thus, based on analysis of the structure-activity relationship of these phytoestrogens and other ER ligands, it was concluded that differences in ligand behavior are primarily related to the 'ligand structure' [21]. Moreover, recognition and interpretation of ligands at the ligand-binding pocket of

ERα have also been ascribed to interplay between the shape of the ligand and the amino acid (aa) residues that support ligand accommodation at the receptor binding cavity.

Given the structural features of ERα agonists, such as DES and E₂, a hydroxyl group in the phenolic A ring appears essential for agonist recognition and activity [22]. Not surprisingly, biological studies with RSVL have also indicated that its 4'-hydroxyl group is absolutely required for its pharmacological actions such as inhibition of cell proliferation and scavenging of free radicals [22–24]. Considering the molecular modeling determinants of DES and RSVL, it appears they share two phenolic rings (A and B) and a stilbenic bond. However, the B ring for DES carries only one 4-hydroxyl group versus two (3,5)-dihydroxyl groups for RSVL (fig. 3). Therefore, other orientations for RSVL at the binding domain have to be evaluated, especially because a crystal structure for RSVL-ERα complex is lacking. In total, four possible orientations for RSVL can be generated by rotation about the stilbenic bond and/or exchanging location of monophenolic and diphenolic rings (fig. 3). We employed the DES-phenolic ring A as a guiding tool to correctly position these RSVL models at the ERα binding domain [13, 16]. The energy-minimized models were comparatively studied based on their features of accommodation at the ERα binding pocket.

Table 1 demonstrates that the RSVL-a mode had superior hydrogen bonding profiles and showed the lowest

Fig. 4. a Flat-ribbon presentation of ER α in complex with RSVL-a (space-filling). The defined residues (dark structures) are those involved in H bonding with RSVL-a. b Binding mode of (E)-RSVL-a docked and minimized in the ER α binding pocket showing residues involved in its recognition. Dashed lines indicate H-bond formation and numbers indicate their length in ångström.

energy of interaction with the receptor (fig. 4). Model 'RSVL-b', resulting from rotation around the stilbenic bond, had no capacity to form H bonds with His524 or Leu525 (fig. 5). The two latter residues, as established by mutation studies, are pivotal for ligand interaction and recognition [25]. Also, the energy of interaction of RSVL-b with the receptor (−8.3 vs. −16.3 kcal/mol for RSVL-a) suggests less favorable accommodation within the receptor binding pocket. The other two orientations, RSVL-c and RSVL-d, resulting from switching the position of rings A and B, lacked the ability to interact with Arg394, a crucial ligand-binding residue [26]. Besides, as reflected by their energy of interaction, RSVL-c and RSVL-d were poorly accommodated within the receptor binding pocket (table 1). Apparently, exchanging rings A and B increased the steric repulsion and adversely affected their complementarity with the receptor. These studies suggest that RSVL-a mode led to the most favorable interaction with the ER α . Likewise, alignment studies with DES-ER crystal structure indicated that RSVL-a

Table 1. Calculated ligand binding interaction energy at the ER α binding cavity (kcal/mol)

Ligand	E_{ligand}	E_{complex}	$E_{\text{interaction}}$
RSVL-a	−3.7	−298.0	−16.3
RSVL-b	−3.7	−290.0	−8.3
RSVL-c	−3.7	−285.0	−3.3
RSVL-d	−3.7	−283.0	−1.3
DES	−10.5	−298.6	−10.1

$$\text{Energy of ligand free receptor} = -278.0 \text{ kcal/mol } E_{\text{interaction}} = E_{\text{complex}} - (E_{\text{ligand}} + E_{\text{receptor}})$$

orientation is far superior to the rest of the RSVL orientations. Accordingly, the modeling characteristics of RSVL-a and DES have been comparatively evaluated. For both ligands, the 4'-hydroxyl group contributes a hydrogen bond with the receptor's glutamate (Glu353). Moreover,

Fig. 5. Binding mode for RSVL-b docked and minimized in the ER α binding pocket, showing residues involved in its recognition. Dashed lines indicate H-bond formation and numbers indicate their length in ångström.

this same hydroxyl group is additionally recognized by the side chain of the conserved arginine (Arg394), which correctly orients and braces the ligand at the binding pocket of the receptor. Histidine (His524) is a third important catalytic residue which interacts, via its delta nitrogen, with the 4-hydroxyl group of the B ring of DES or the 5-hydroxyl group of RSVL (fig. 2 and 4, respectively). This binding mode of RSVL and DES with the catalytic triad aa is similar to that observed for the prototype E₂ (fig. 6) [26]. Thus, it appears that all hydroxyl groups from all ligands were actively engaged in H bonding (fig. 2, 4, 6). Uniquely, however, RSVL-a developed three additional H bonds with Gly521, Leu525 and Met343. Evidently, such additional recognition for RSVL-a results from the altered orientation of its B ring hydroxyl groups (3,5-OH), as compared to that of DES (4-OH, fig. 7). As established by mutation and receptor binding studies with E₂, Gly521 and Leu525 have been identified as key components for interaction and/or interpretation of ligands at the ER α binding cavity [25]. These residues were hydrogen-bonded to the 5-OH group of RSVL's B ring. The pocket residue, Met343, has been identified as a merely hydrophobic van der Waal contact point for E₂ [26]. Currently, this residue was recognized by RSVL through H bond interaction, due to the presence of 3-OH group in its B ring. This latter interaction further constitutes a unique

recognition mode for RSVL that was not observed for other ligands modeled at the binding domain of the ER α receptor [27]. However, the impact and implications of this additional interaction remain to be substantiated via experimental receptor mutation studies. Lastly, RSVL exhibited a more favorable energy of binding than DES (–16.3 vs. –10.1 kcal/mol). This is likely conferred by the differential ligand interaction with the pocket aa residues. Previous molecular modeling studies with ER ligands suggested that more favorable interaction energy associates with partially antagonistic rather than purely agonistic activity, which conforms to the present observations as well as the behavior of RSVL in ER α -responsive systems [10, 18].

Although the present study is apparently a merely computer-based evaluation, its biological relevance is warranted. That is, it relied on a documented three-dimensional topography of the receptor and actual structure/geometry of its aa pocket residues. The present notion that RSVL mimicked the binding of DES to the catalytic triad aa (His524, Arg394 and Glu353) conforms well to its actions as an agonist in estrogen-free systems. On the other hand, the additional recognition of RSVL by three other ER α pocket residues (Gly521, Leu525 and Met343) may influence the stability of the formed receptor-ligand complex, and the readiness of this complex to reach the nucleus or to interact with the E₂ nuclear machinery. This view is reinforced by experimental findings on activation of ER α in human HepG2 liver and U2 osteogenic cancer cells. Thus, RSVL was an agonist that stimulated ER α -dependent luciferase activity in U2 cells transfected with ER activation factor-1 (ER-AF1), but not with ER-AF2. Another phytoestrogen, naringen, activated only HepG2 cells transfected with ER-AF1. In HepG2 cells, only RSVL attenuated the E₂-evoked, ER α -mediated luciferase reporter activity [21]. Accordingly, it is tempting to hypothesize that coexistence of two ligands that variably bind the pocket aa residues could adversely affect the binding and responsiveness to the purely agonistic ligand, E₂. These perspectives may well agree with the observed agonist/antagonist behavior of RSVL in some biological systems [28]. Indeed, site-directed mutations of aa residues of the ER α pocket clearly demonstrated that antagonists like hydroxytamoxifen display preference to bind some pocket residues other than those best recognizing the full agonist E₂. However, some aa residues were inevitably required for actions of both agonists and antagonists [25], as similarly demonstrated in the present work. In this context, it is worthwhile mentioning that RSVL is not merely an ER ligand, as it also elicits a myriad of actions

Fig. 6. a Flat-ribbon presentation of the crystallographic structure of E₂-ER α -LBD showing E₂ (space-filling) and the catalytic triad residues (His524, Arg394 and Glu353, dark-stick) at the ER binding domain. b Crystal structure of E₂ at the ER α binding pocket showing H-bonding interactions with the catalytic triad residues (dashed lines). Numbers indicate the length of HB in ångström.

Fig. 7. Superposition of the X-ray structure of DES and the energy-minimized RSVL at the active site of ER α . The overlay demonstrates that DES and RSVL have common binding to the catalytic aa triad. Besides, RSVL exhibited inimitable interaction with other key residues (Gly521, Leu525 and Met343). Dashed lines represent HB interaction of RSVL, and solid lines represent those of DES.

pertinent to other cellular effectors, including enzymes [1] and cell cycle checkpoints [29]. Thus, RSVL was established as a versatile agent that can act by both ER-dependent and -independent mechanisms, occasionally even in the same biological system [29].

Conclusion

Altogether, the present study highlights major differences in the modeling characteristics of pure estrogen agonist DES and the biologically active ER agonist/antagonist RSVL; these variations may entail distinct patterns of ligand recognition 'footprinting' that subsequently dictate the ligand's biological actions.

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