Binding Affinity of 17-β-ethinylestradiol to Normal and Mutant Types of Estrogen Receptors

An in silico evaluation

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Estrogen receptors (ERs)are nuclear transcription factor receptorsthat play important roles in gene expression and cell cycle regulation. Because of their ligand-activated signaling implication in carcinogenesis,ERs are extensively researched as protein targets for anti-cancer drug discovery active in certain types of tumors. However, a major drawback is the emergence of wild type tumors withoverexpressed mutant variants of ER, which become resistant to estrogen inhibitor drugs. Herein we studied the binding mode and affinity of the semisynthetic estrogen agonist, 17-β-estradiol (E2) in normal and mutant variants of ERs, by means of molecular docking. Our results showed a small decrease in binding affinity, recorded in mutant variants of ERα and a change in the binding interactions formed when the compound wasdocked in an agonist-bound conformation of the ERα . Nevertheless we concluded that even if the binding affinity showed a small decrease in the case of mutant type receptors,E2 potency towards ERáwon't register a downward trend.

Keywords: ethinylestradiol, estrogen receprors, mutantion, docking

Estrogen receptors (ERs) represent ligand-activated transcription factors belonging to the larger nuclear hormonereceptor family. There are 2 types of ERs, namely, ERα and ERβ, both being expressed in a vast variety of tissues and cell types [1]. ERs are involved in estrogen mediated signaling pathways which play an important role in gene expression modulation and cell cycle regulation. The two abovementioned receptor classes have opposite biological effects if co-expressed, ERβ antagonizing the mediated effects of ERα activation [2]. ERs expression through ligand mediated signaling is widely known to promote carcinogenesis in some types of tumors such as breast and colorectalcancer.Ligand binding to the two receptors (ERα/ERβ) induces ER dimerization and further nuclear translocation andbinding to specific target genes in hormone receptor elements (HREs) and other DNA regulatoryelements[3,4].The ERα which is significantly expressed in ER positive breast cancers is being extensively studied as a target for the development of inhibitors as novel anti-cancer agents [5,6]. An increasing concern in the utilization of ERα inhibitors is represented by the installation of drug resistance in certain types of breast tumors correlated with the expression of mutated ERα isoforms. In the most frequent ER mutations, such as Y537S and D538G, due to the conformational structural change produced by the amino acid residue modification, different ligand-binding domain overall topology was encountered, which influenced the binding affinity of inhibitory drugs and subsequently their antiproliferative activity [7,8]. ERs are also known to be associated with increased melanocyte and melanin production in human skin. ERβ is the most frequently expressed ER in human melanoma playing an important role in various skin cells and melanocytic tumors [9].

Ethinylestradiol (EE2) is s semisynthetic alkylated estradiol (fig. 1) acting as a potent ER agonist, widely used as contraceptive oral medication [10].An increasing concern is that patients continuously using this contraceptive medication are often associated with high risks of developing several types of cancer.

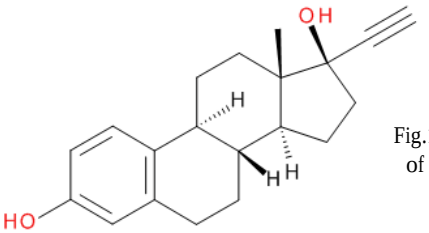

Fig.1. Molecular structure of 17-b-ethinylestradiol (EE2)

Molecular docking is a tool used for the rational discoveryof new active molecules or for providing a better understanding of various molecular mechanisms by offering information regardingthe binding particularities of certain molecules to specific protein targets.

The aim of our current study is to compare the binding mode and affinity of EE2 to the ERα, some of its important mutant isoforms, and ERβ as well. The recorded information could be useful in understanding specific aspects regarding the connection between EE2 biological activity and certain ERs signaling correlated pathologies.

Experimental part

Materials and methods

Protein structures used, were available from the RCSB Protein Data Bank [11]. For the purpose of this study structures corresponding to ERα (normal and available mutantisoforms) and ERβ(PDB ID's: 1A52, 4Q50, 5T1Z, 5W9D, 3OLS) were prepared as suitable targets for molecular docking using Autodock Tools 1.5.6. From each structure, water molecules, metallic atoms and the co-crystalized ligand (if present) were removed, after which Kollman charges and polar hydrogen atoms were added for each protein. Targets were saved as .pdbqt files. EE2 structure was processed using PyRx's Open Babel module, for the purpose of molecular geometry optimization. Ligand structure was afterwards converted to the .pdbqt format. The co-crystalized ligand, 17-β-estradiol (E2) of 1A52 (ERα) and 3OLS (ERβ) was removed from the protein structures, prepared as suitable .pdbqt ligand and re-docked in the structures, for the purpose of comparing obtained

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binding energies of E2 and EE2 with known tested affinities for the two structures and for the validation of the employed method.

Molecular docking was carried out with the PyRx software (Version 0.8) using Vina's scoring function [10]. Molecules were docked in the estrogen ligand binding domain of each protein structure, using default docking parameters. Recorded scores for docked molecules were given as free binding energy values (kcal/mol). Ligand-protein binding particularities were analyzed using Accelerys Discovery Studio 4.1 (Dassault Systemes Biovia).

Results and discussions

Binding energy scores, attributed by Vina, for EE2 and E1 after docking in human non-mutant structures of the two ERs are presented in table 1.

Table 1

BINDING ENERGY SCORES FOR ETHINYLESTRADIOL (EE2) AND ESTRADIOL (E2) DOCKED IN NON-MUTANT STRUCTURES OF ERα AND ERβ

Receptor (PDB ID)	Docked ligand	Binding energy (ΔG) of best ligand conformation (kcal/mol)
ERα (1A52)	E2	-10
ERβ (3OLS)	E2	-11.3
ERα (1A52)	EE2	-9.3
ERβ (3OLS)	EE2	-8.1

A comprehensive study by Shityakov et al. regarding the correlation between predicted protein-ligand free binding energies (ΔG) using Autodock and Vina, and compound activities, suggested that ΔG values should be below a threshold value of -6 kcal/mol in orderfor the predicted activity to be significant [12]. As results show, calculated binding energies of the two docked compounds are below the above mentioned threshold value, which clearly correlates with their estrogenic activity. Literature shows that in terms of affinity towards ERs, E2 is more potent then EE2 when binding to ERα, but in the caseof ERβ, EE2 exhibits a higher affinity [13,14].In the case of ERα docking results, we obtained a lower ΔG valuefor EE2, meaning EE2 should have a higher affinity for this receptor. Nevertheless, literature shows that even if IC50 values for E2 related to ERα are lower than EE2 IC50 values, the difference is not that significant, both concentrations being in the 10^{-10} M range [13]. Since docking programs have empirical scoring functions built to predict binding conformations and formed interactions, a difference of 0.7 kcal/mol is not significant enoughto compare the affinity of two different compounds.

From the binding mode analysis using the highest affinity conformations of E2 and EE2 in both ERs we notice that EE2 accommodations in both ERα and ERβ binding domains are extremely similar to that of E2 (figs. 2 and 3).

In both cases the phenolic -OH is responsible for hydrogen bond (HB) formation with residues Glu353, Arg394; however, in the case of E2,an additional HB is formed between the alcoholic -OH and His524 (fig. 2A,B - highlighted areas). When superimposing the two docked molecules a topological similarity canbe observed. Both

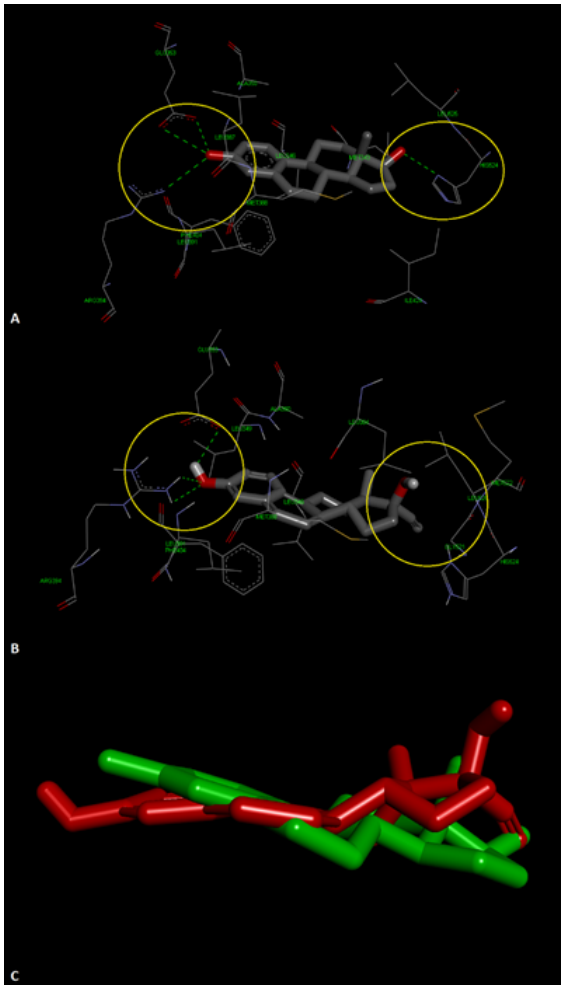

Fig.2. Binding mode of E2 (A) and EE2 (B) in the active site of ERα(PDB ID: 1A52) and superimposed docked structures of E2 (green) and EE2 (red) (C); HB interactions (dotted green lines) formed with Glu353, Arg394 (A, B) and His524 (A). Hydrophobic interactions are not depicted for better image clarity

molecules are oriented in a similar manner and the co-planarity of the two structures is relatively high (fig. 2C).

The same features and similarities are also present when the two molecules were docked in ERβ (fig. 3). In both cases the phenolic -OH is responsible for HB formation with residues Glu305, Arg346 but, in the case of E2,an additional HB is formed between the alcoholic -OH and His475 (fig. 3A,B-highlighted areas). The structure orientation similarity,between E2 and EE2 is present here as well (fig. 3C).

Results for EE2 ΔG values docked in mutant isoforms of ERα are presented in table 2. We selected the ΔG values of the best 4 conformations of EE2 for each case in order to achieve a better assessment of the affinity modification tendencies.

As results show, there is an observable decrease in binding affinity (higher ΔG values) related to the mutated forms of ERα compared to the non-mutant structure, but the decrease is not significant enough to conclude that there is a drop in EE2 activity involving mutated ERα mutant types. Even if the structural change in amino acid residues is correlated with protein overall conformational change[7,8], the modified amino acid residues involved in the mutant ERα 3D structures used in our study (Tyr536, Tyr537, Asp538, Cys 381, 417, 530) were not directly involved in the resulting interactions of EE2 in the active site of the protein (fig. 4). These aspects could explain the small difference noticed in the predicted binding activities

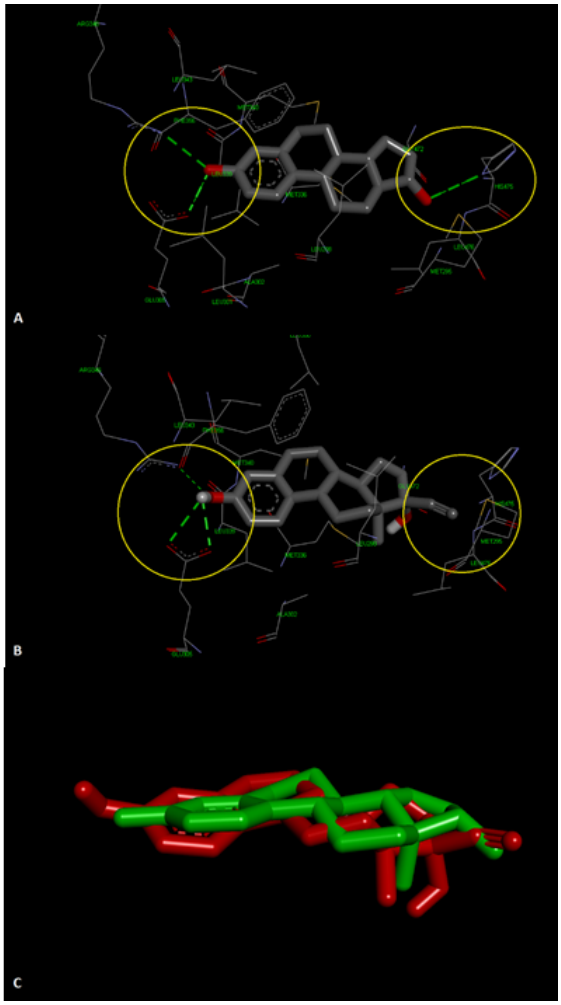

Fig.3.Binding mode of E2 (A) and EE2 (B) in the active site of ERβ (PDB ID: 3OLS) and superimposed docked structures of E2 (green) and EE2 (red) (C); HB interactions (dotted green lines) formed with Glu305, Arg346 (A, B) and His475 (A). Hydrophobic interactions are not depicted for better image clarity

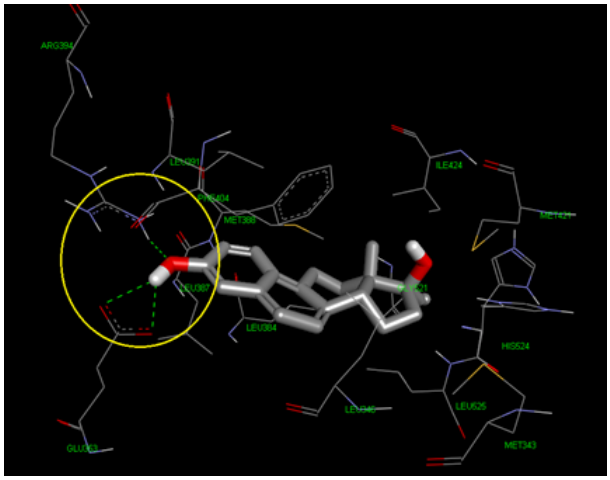

Fig. 4. Binding mode ofEE2in the active site of ERα Y537S mutant (PDB ID: 5T1Z); HB interactions (dotted green lines) formed with Glu353, Arg394. Hydrophobic interactions are not depicted for better image clarity

of EE2, but we can state that a loss of EE2 activity related to mutant forms of ERá cannot be considered since ΔG obtained values were below the -6 kcal/mol threshold.

An interesting feature was recorded in the docked conformation of EE2 in the active site of 2QXS (fig. 5). This structure represents a mutated form of ERα with a different

Table 2

BINDING ENERGY SCORES FOR ETHINYLESTRADIOL (EE2 - BEST 4 CONFORMATIONS)DOCKED INMUTANTPROTEIN STRUCTURES OF ERα

PDB ID	Mutation	Ligand docking conformation	BInding energy (ΔG) (kcal/mol)
1A52	-	EE2_model 1	-9.3
		EE2_model 2	-8.1
		EE2_model 3	-7.9
		EE2_model 4	-7.9
2QXS	Y536S	EE2_model 1	-8.3
		EE2_model 2	-7.9
		EE2_model 3	-7.9
		EE2_model 4	-7.8
4Q50	D538G	EE2_model 1	-9.2
		EE2_model 2	-8.2
		EE2_model 3	-7.9
		EE2_model 4	-7.7
5T1Z	Y537S	EE2_model 1	-9.3
		EE2_model 2	-8.9
		EE2_model 3	-8.3
		EE2_model 4	-7.9
5W9D	C381S, C417S, C530S	EE2_model 1	-8.4
		EE2_model 2	-7.4
		EE2_model 3	-7.2
		EE2_model 4	-7

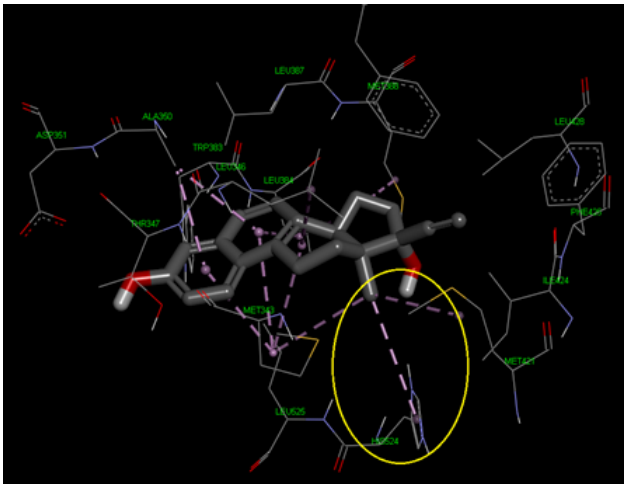

Fig.5. Binding mode of EE2in the active site of ERα Y536S mutant (antagonist bound conformation) (PDB ID: 2QXS); hydrophobic interaction (purple dotted lines) formed with Hys524, due to the conformational change of the active domain

conformation due to the binding of an estrogen antagonist. As we can notice the amino acid residues Glu353 and Arg394 are no longer within the range of the -OH phenolic group thus rendering the formation of HB impossible. At the other end, His524 has shifted its position due to the conformational change and now forms a hydrophobic interaction with the nearest methyl group (fig. 5-highlighted region). As a result, it would seem that in mutant forms that adopt the specific antagonist binding conformation the usual binding mode of EE2 is significantly affected. This could also be correlated to the fact that in wild type

mutant ERα expressed tumors, inhibitors show less potency due to the tumor acquired resistance [15].

Collectively, these findings indicate that EE2, will still retain its affinity for the ER and subsequently will induce the expected effects when bound to a mutant variant of the receptor, as long as the amino acid residues do not directly interfere with the ligand binding domain.

Conclusions

In the present work we evaluated the binding affinity and particularities of estrogen receptor agonist, 17-β-ethinylestradiol, by means of molecular docking against normal and mutant type ER. Our results indicated that despite the fact that mutant variants of ER suffer conformational change due to amino acid changes, obtained binding energies of E2 did not decrease significantly. It is probable that the conformational changes don't affect agonist binding, only when the amino acid changes directly affect the ligand binding domain.

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