Aqueous solution interactions with sex hormone-binding globulin and estradiol: a theoretical investigation

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Abstract Sex hormone-binding globulin (SHBG) is a binding protein that regulates the availability of steroid hormones in the plasma. Although best known as a steroid carrier, recent studies have associated SHBG in modulating behavioral aspects related to sexual receptivity. Among steroids, estradiol (17 β -estradiol, oestradiol or E2), documented as the most active endogenous female hormone, exerts important physiological roles in both reproductive and non-reproductive functions. In this framework, we employed molecular dynamics (MD) and docking techniques for quantifying the interaction energy between a complex aqueous solution, composed by different salts, SHBG and E2. As glucose concentration resembles measured levels in diabetes, special emphasis was devoted to analyzing the interaction energy between this carbohydrate, SHBG and E2 molecules. The calculations revealed remarkable interaction energy between glucose and SHBG surface. Surprisingly, a movement of solute components toward SHBG was observed, yielding clusters surrounding the protein. The high energy and short distance between glucose and SHBG suggests a possible scenario in favor of a detainment state between the sugar and the protein. In this context, we found that glucose clustering does not insert modification on binding site area nor over binding energy SHBG-E2 complex, in spite of protein superficial area increment. The calculations also point to a more pronounced interaction between E2 and glucose, considering the hormone immersed in the solution. In summary, our findings contribute to a better comprehension of both SHBG and E2 interplay with aqueous solution components.

Keywords Electrolyte solution · Sex hormone bind globulin · Molecular dynamics · Docking · Estradiol · Glucose

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1 Introduction

The representative effects of electrolytes on biomolecules have been explored since the pioneering studies of Hofmeister on protein precipitation induced by salts [1]. In living organisms, a huge family of pumps, channels, and receptors regulate ionic levels [2]. Moreover, it is well known the modifications on solubility and melting temperature of proteins promoted by ionic species [3]. Regarding physiological systems, several experimental investigations have revealed important biophysical aspects of the ionic interactions with hormones. Within this scope, Peggion et al. explained how calcium interacts with gastrin family peptides in two different media made by trifluoroethanol solution and water [4]. It is also interesting to mention a thermodynamic study on the interaction between magnesium and human growth hormone, carried out by Saboury et al., showing a protein thermal stability increment [5]. Furthermore, reports, assessing modifications in electrolyte composition of cervical mucus, provided a correlation between estrogen levels with chlorine and sodium [6]. On the other hand, despite the wealth of empirical studies involving ionic interactions with proteins, valuable information has also been harvested using simulations [7]. In recent years, the extensive development of analytical and numerical methods allowed application of theoretical methodologies as molecular dynamics (MD) and docking techniques in different branches of science. These approaches, created for translating physical laws that represent biological systems, supply a faithful mechanistic representation of experimental results. A remarkable advantage using *in silico* approach is the opportunity for preparing simulations based on difficult experimental maneuvers. For this reason, simulations emerge as a useful approach to provide insight into the intermolecular complex structure target-ligand, predicting free energy bindings by assuming scoring functions [8, 9]. In this context, computer experiments successfully assessed several physical properties relative to protein interactions with aqueous solution elements at different physicochemical conditions [10, 11]. Simulations also allowed better comprehension about sodium and chloride propagation near the lysosome surface, elucidating the role of calcium in the dynamics and global structure of parvalbumin in aqueous solution [12, 13]. Furthermore, computational investigations demonstrated the influence of zinc on SHBG-steroid binding properties, such as lysozyme properties modified by saline solution [14, 15]. Besides their well-known interactions with ions, proteins also form covalent bonds with a variety of sugars in reactions known by glycation and glycosylation [16]. Both processes, commonly observed in diabetes metabolic derangements, characterize themselves by high glucose concentration in the blood, differentiate from each other by absence (glycation) or presence (glycosylation) of enzymatic controlling. In this sense, an important effect of hyperglycemic environment is the promotion of structural and functional modifications in serum albumin and myoglobin [17, 18].

Daughaday firstly reported the existence of SHBG in the 1950s, but only after the investigations by Mercier et al. the seminal findings were fully accepted [19]. SHBG binds to steroids in the plasma modulating the hormone availability in most groups of vertebrates including mammals, reptiles, amphibians, and fishes [20]. Human SHBG is a homodimeric glycoprotein, produced and secreted by the liver, placenta, and testes, where each monomer consists of 373 amino acids residues, existing as a 90-kDa protein containing a single binding site [21]. Crystallographic analysis at 1.55 Å showed important details of the steroid-binding site and quaternary structure of the dimer [22]. Recent studies have associated SHBG with

other important physiological roles associated to sexual receptivity and metabolic disorders such as diabetes and obesity [21, 23–26]. Among steroids bound to SHBG, one may highlight E2, which binds with a dissociation constant in nanomolar range [27, 28]. The E2 molecule has a low hydrophilic degree, molecular volume of $245 \text{ \AA}^3$, and apolar surface area of $261 \text{ \AA}^2$, being a final product of several enzymatic reactions including cholesterol and testosterone catabolism [29]. Since its discovery, efforts have been devoted for elucidating the E2 physiological functions, especially those related to molecular machinery of reproduction, enabling wide range of drugs development [27]. However, further investigation showed that E2 is not restricted to the peripheral reproductive system. In fact, E2 is associated to diabetes, inflammation, and breast tumors, among others [30–33]. The brain is a target for hormonal action synthesized from the neuronal metabolism as well. As a consequence, E2 exerts notable influences on mechanisms relative to memory formation and neuroprotection, since it modulates the dimorphism in brain morphology [34–36].

In this work, the foremost purpose is to combine MD and molecular docking to investigate three main issues: (1) how dynamics and structural changes of SHBG are affected by electrolyte solution; (2) a possible sugar agglomeration close to the protein once the glucose content, adopted in the present work, is within the diabetic range; (3) the consequences of such clusters on the SHBG-E2 binding energy.

2 Materials and methods

2.1 Preparation of protein and ligand for docking

Calculations relative to the E2 docking on SHBG were based on three-dimensional structure of SHBG in complex with dihydrotestosterone (DHT) obtained from the Protein Data Bank (PDB code 1KDM). The structure of E2, COD-CID 5757, was obtained from PubChem. Hydrogen atoms were added and bonded using the AutoDock Tools 4.2 with partial charges calculated using empirical force field MMFF94 [37]. The binding site selected for docking was formed by the residues Thr40, Ser42, Phe56, Asp65, Phe67, Leu71, Leu80, Asn82, Val105, Met107, Val112, Ser128, Met139, and Ile141, Wat 364, and Wat 369. To supply new binding geometries for ligand and side chains, selected residues were considered flexible. Besides, the selection itself was accomplished by a manual procedure based on knowledge of amino acids composing the active site with ligand molecule (E2) obtained from PubChem database (pubchem.ncbi.nlm.nih.gov/) at the “SDF” file format [38–40]. Open Babel Software version 2.3.1 converted ligand representation into PDB format [22]. Next, all hydrogens and partial charges were added applying the empirical force field MMFF94 [41]. Finally, visual inspection through PyMOL viewer version 1.7.x was performed for correcting eventual structural errors on SHBG structure [42].

2.2 Simulation redocking

The present work used AutoDock Vina for adjustment of the docking parameters and removal of all water molecules from SHBG, except those belonging to the active site, because they may occupy conserved positions influencing the ligand recognition process [43]. As previously mentioned, a three-dimensional structure of SHBG in complex with DHT was used in the

study (obtained from the Protein data Bank code 1KDM). DHT substrate was further extracted from SHBG, recoupling it again to the protein through redocking simulation. This procedure is a process by which a ligand taken from the structure of a complex with a receptor is docked to the “induced-fit” form of the receptor. This method is often performed to verify the docking parameters, specified in the input file. Furthermore, a 3-D simulation box, considering 50 Å in each direction, was defined around the binding site of the protein, with center under DHT coordinates -5,491, -39,238, 32,056. The most accurate results in terms of redocking were obtained and compared with DHT crystallography position, as deduced by superposition of the two structures (Fig. 1). Table 1 shows the best parameters found in redocking (RMSD 0.49 Å) applied to adjust E2 docking into SHBG. The energy range specifies the maximum number of binding modes to output, i.e., the maximum energy.

2.3 Protein-ligand docking

The achievement of E2 (supplementary material, Figure S1) docking into SHBG employed AutoDock Vina with all complexes visualized in PyMOL [42, 43]. The best binding modes procedure obeyed two main steps: (a) splitting of identical solutions in terms of conformations, where the results of low energy were separated for further visual comparison with DHT crystallographic structure position; (b) use of both lower RMSD and energy values to generate the model to SHBG-E2 complex and its subsequent optimization with Steepest Descent method, enabling improvement in the accuracy of binding mode (Fig. 2).

Fig. 1 Overlapping of the DHT in redocking simulation. Docked DHT is colored in *green* and *red* (image created using Pymol software)

Table 1 Parameters found in redocking used to set E2 docking into SHBG

Parameters	Value
Energy range	10
Num modes	20
Exhaustiveness	8
Seed	-150

Energy range (kcal/mol) describes the maximum energy difference between the best and worst binding mode; “Num modes” is the parameter associated to the maximum number of binding modes; “Exhaustiveness” is related to thoroughness of search, roughly proportional to time; “Seed” is associated to a random number generator

2.4 Molecular simulation

The simulation was built using Gromacs 5.0.2 package adopting the Charmm27 force field with the structure taken from the SHBG-E2 complex (Fig. 2) obtained from docking procedure [44]. E2 topology and salts were obtained from Web SwissParam server with partial charges assigned of the force field MMFF94 [45]. To perform simulations all crystallized water molecules were excluded from the complex. Next, the LINCS algorithm was applied for all bonds within the complex allowing 2-fs time steps, whereas the SETTLE algorithm was adopted for water molecules bonds [46, 47]. The cut-off to the non-bonded interactions was 2 nm with electrostatic interactions computed by using the particle mesh Ewald method [48]. Based on previous investigations, the temperature was maintained constant through the weak couple of V-rescale with a time constant of 0.1 ps, being the pressure equilibrated at 1 bar through Parrinello–Rahman method using a pressure of 2 ps time constant relaxation [49, 50]. The present work considers SHBG and E2 immersed, at the mammalian physiological temperature (37 °C), into a saline solution with constituents similar to a Ringer solution. Complex was soaked in an octahedron box of volume 712.03 nm³ with water (700 mM)

Fig. 2 3D-Structure of SHBG
(image created using VMD
software)

TIP3P type, KCl (12.4 mM), CaCl_2 (27.1 mM), MgCl_2 (16.6 mM), NaCO_3 (44.5 mM), $\text{Na}_2\text{H}_2\text{PO}_4$ (16.8 mM), and glucose (24.5 mM) with margin of 1.2 nm between SHBG-E2 complex and each side of the box. Non-periodic boundary conditions were applied to the system neutralized by adding 128 mM of NaCl molecules.

The binding free energy between SBHG and E2, calculated by LIE method, assumed two approaches: firstly, considering SBHG-E2 complex followed by a second contemplating E2 in a free protein aqueous media solution. Both systems (bound and free) were equilibrated in two phases: 5 ns in the NVT ensemble followed by the same time in the NPT ensemble. Free and bound systems were simulated during 450 ns, at temperature of 310 K and pressure of 1 bar with initial velocity generated from the Maxwell–Boltzmann distribution. We calculated bound and free energies of molecular complex between a protein P and a ligand L by using:

$$\Delta G = G(P : L) - G(P) - G(L), \quad (1)$$

where ΔG represents the binding energy difference between E2 bonded and non-bonded states. Simulation was done on free E2, in the same way as described above, for the SHBG-E2 complex to obtain the van der Waals and electrostatic interaction energies between E2 and surrounding environment. The empirical parameters used to calculate the free energy are $\alpha = 0.348$, and $\beta = 0.176$, chosen after testing several values from literature. The results were compared with those binding free energies obtained by Shanbhag and Södergård, taking the average energies over the frames harvested from all trajectories [51]. Calculation of binding free energy was accomplished by using LIE method due to its faster performance in relation to other methods [52, 53]. This method calculates van der Waals and electrostatic interactions relative to a free and bound ligand, according to the following equation:

$$\Delta G_{\text{bond}} = \alpha \left(\langle V_{l-s}^{\text{vdw}} \rangle_{\text{bound}} - \langle V_{l-s}^{\text{vdw}} \rangle_{\text{free}} \right) + \beta \left(\langle V_{l-s}^{\text{ele}} \rangle_{\text{bound}} - \langle V_{l-s}^{\text{ele}} \rangle_{\text{free}} \right), \quad (2)$$

where the α and β empirical constants represent van der Waals and electrostatic scaling parameters factors, $\langle \rangle$ is the average of the potential energy relative to the free or bonded van der Waals (vdw) and electrostatic (el). The equation also considers the contribution from the ligand-surrounding environment (l-s) [54]. Finally, all protein figures were created employing PyMOL version 1.7.x, while graphs by using Origin (OriginLab, Northampton, MA, USA).

3 Results and discussion

3.1 Complex time evolution

The interaction between side chains and consider solvent is a relevant factor responsible for the protein native structure. Consequently, modification on the solvent composition naturally implies changes in the structural thermodynamics balance [55]. In this sense, due to increment of pH or ionic concentrations, water molecules interact with ions by reducing the water–protein synergism. Thereafter, a decrement in protein–protein interactions induces structural alterations in the side chains of the binding sites and ligand affinity [56]. In the present work, we analyzed the root-mean-square deviation (RMSD) of the SHBG-E2 complex in relation to the optimized structure by inspecting the conformational states. It is important to mention that in the simulations, 5000 steps were employed for extracting the frames necessary to write coordinates to output trajectory. Figure 3 shows conformational states evolution of complex from 0.04 to 0.21 nm, where the first equilibrium

Fig. 3 Root-mean-square deviation (RMSD) of protein (*red*) and E2 (*black*)

state consolidates at 11.5–50 ns. After that, other partial states are observed from plateaus around 68–125 ns (0.20 nm), 200–227 ns (0.20 nm) and, 230–284 ns (0.22 nm), 342–386 ns (0.21 nm), demonstrating several state transitions towards the final native state. The RMSD shows fluctuations because final conformation may still have some free movement, which is important to several physiological mechanisms [57]. As shown in the supplementary material (Figure S2), changes in system energies were monitored where potential U (-1.14×10^5 kJ/mol) and kinetic (1.46×10^4 kJ/mol) are stable from 250 ns. Still according to these calculations the kinetic energy is proportional to temperature, configuring an energetically stable physical system. In relation to E2, fluctuations around 0.02 nm indicate large displacements relative to the initial position. Yet, it is important to mention that water molecules may contribute to promote the system stabilization, facilitating E2 achieves the active site. We have determined the presence of water molecules such as ions surrounding E2, using the radial distribution function:

$$g(r) = \frac{n(r)V}{N\Delta V}, \quad (3)$$

where $n(r)$ is the average number of atoms in a certain volume. Figure 4 illustrates a representative $g(r)$, made from 350 ns of trajectory, of the ionic and water molecules distributions placed at distance r from E2. Peaks in $g(r)$ indicate a larger solvent molecule density in the spherical shell at $r = 1.8$ nm, in relation to the ligand. It is worth mentioning that water molecule dynamics exhibits anomalous behavior in the presence of different electrolytes [58]. In this ambient, significant enthalpic driving force arises, since the local electric field increment of this association restricts the mobility of nearby water molecules [59]. The presence of ions in the system induces ion–water hydrogen bonds to form a network, confining ion–water into a layer thickness surrounding protein [60]. Additional data are given in Figure S3 (supplementary material), where it was confirmed the absence of artifacts given by water and ion density. Furthermore, there were no hydrogen bonds between water molecules or residues of the side chains and E2 (data not shown). For calculations related to hydrogen bonds, one assumed $r \leq 0.35$ nm and $\alpha = 30^\circ$, where r is the minimum cut-off radius and α is the minimum angle required for hydrogen bond formation. The absence of water molecules, salts, or ions near the ligand assures the admission that at least non-polar and electric

Fig. 4 Radial distribution function of shells of ions and water surrounding E2

interactions must be responsible for the SHBG-E2 binding. Still, according to Fig. 4, the separation between the first peaks of ion-water provides insight about the predominance of water molecules around the ion. In this sense, our results are similar to those obtained by Rajamani et al. [61]. The packing of water molecules surrounding E2 suffer influence of charge density of ionic species for r (nm) < 2.7. On the other hand, for r (nm) > 2.7 the density can achieve uniformity, that is, equivalent to a diluted state.

3.2 Binding site

It is suggested that conformational changes on SHBG are partially due to the salts over its surface. Undoubtedly, the simulation shows most ions interacting with water molecules with SHBG. Additionally, it is also important to highlight a clear glucose cluster formed near the protein (Fig. 5). Therefore, one could hypothesize that dynamic binding site and its interaction with E2 would produce changes in conformational states within the protein. Ionic compaction effect over SHBG was inspected on the accessible surface area to solvent (SASA) in relation to the optimized structure, which increased from 80 to 90 nm² (standard deviation $\pm$ 0.002 nm²) (Fig. 6). This SASA increment is a consequence of clustering formation on the SHBG. Nonetheless, the same calculations relative to the SHBG binding site residues and E2 did not show any significant SASA modification during the simulation, maintaining in 26.1 and 5.1 nm², respectively. Finally, we investigated the protein compaction, showing the increment of protein radius of gyration, from an initial value of approximately 1.44 nm to a final value (average) at 1.53 nm (Supplementary Material, Figure S4).

3.3 Energetic analysis

This section shows the results of the energy associated to SHBG and E2 conformational changes given by the electrostatic (EL) and Lennard–Jones (LJ) calculations. We present the results in Table 2, where the electrostatic potential dominates the interactions giving stabilization for SHBG-E2 complex. However, LJ does occur in an opposite manner, at least for monovalent ions, but it is important to emphasize that in spite of some regions of SHBG

Fig. 5 Representative snapshot of simulation at 310 K, where ions are represented as *spheres* and presents a magnified size for clarity. Water molecules are shown as bonds (red/white), Na^+ (yellow), Ca^{2+} (purple), K^+ (green), Mg^{2+} (cyan), Cl^- (violet), CO_3^{2-} (silver), PO_4^{2-} (blue), and glucose (orange) as sticks. E2 molecule is represented as sticks colored in red (image created with VMD software)

capture ion and glucose it does not necessarily imply in salts motion decrement. This behavior is credited to small modifications in atomic positions, strong enough for producing prominent fluctuations in LJ component that affect the potential stabilization.

Certainly, the present calculations highlighted a stronger SHBG interaction with glucose. This specific finding motivated the analysis of the glucose clusters formation on the protein in the last 200 ns of simulation. The strategy consisted of placing molecules into a range of 0.2 nm, meaning that only glucose within this region is defined as belonging to the same agglomeration. The cluster position was found to be apparently linked to its size, where the largest fraction of aggregate molecules is formed (on average) by 15 glucose molecules. The

Fig. 6 Fluctuation of solvent accessible area (SASA) for binding site (red) and E2 (black). The inset shows SASA fluctuation of protein

Table 2 Electrostatic and Lennard–Jones interactions between SHBG and aqueous solution components

Energy (kJ/mol)	Ca ²⁺	Cl [−]	K ⁺	Mg ²⁺	Na ⁺	CO ₃ ^{2−}	PO ₄ ^{2−}	Glucose
Coulomb (SR)	− 1234.7 ± 0.27	− 733.8 ± 0.1	− 246.75 ± 0.07	− 732.00 ± 0.02	− 1534.3 ± 0.2	− 1271.9 ± 0.1	− 360.74 ± 0.07	− 1620.0 ± 0.2
LJ (SR)	127.18 ± 0.03	18.84 ± 0.02	21.25 ± 0.01	47.51 ± 0.01	156.84 ± 0.03	− 175.29 ± 0.03	− 119.34 ± 0.17	− 645.09 ± 0.04
Distance (nm)	0.20 ± 0.00	0.20 ± 0.00	0.25 ± 0.00	0.19 ± 0.00	0.20 ± 0.00	0.14 ± 0.00	0.15 ± 0.00	0.15 ± 0.00

Standard deviations were omitted due to their very small values

interaction further quantified between results for simulations concerning E2 with aqueous solution components (Table 3). According to these results, the binding of small ions to E2 is less intense when compared with glucose values. In addition, for this hormone, the nonpolar contribution is the most important factor in the interaction with SHBG. Indeed, electrostatic interaction energy is estimated to be attractive (-0.027 kJ/mol) in relation to closest ions from E2. This behavior reveals an interactive balance emerging as an important function in the folding states of the binding site, where results also indicate invariability of configurations; although it has been detected, some points of considerable fluctuations on the trajectories. Indeed, the formation of two hydrogen bonds was identified between OH (E2) atom with the CA atom (THR40) and OH (E2) with CA (ASP65), which contribute to E2 stabilization (Fig. 7). Table 4 shows EL and LJ interaction potential components that compose ΔG calculated by using Eq. (1). The non-polar term is associated with ligand size, and apolar surface represents its hydrophobic capacity for effect with the binding site [62]. Finally, in this work, the ΔG obtained by LIE method was -54.68 ± 0.01 kJ/mol whereas the dissociation constant (K_d) obtained was 4.7×10^{-10} M.

3.4 Further remarks

The present investigation characterized how aqueous solution interacts with SHBG and E2 as well as SHBG-E2 complex itself. To make an approximation with an artificial physiological environment, it was considered a physiological temperature such as in a saline environment constituted by components found in the plasma of mammals. With this purpose, relative to the SHBG interplay with aqueous constituents, the calculations provided for all ionic species distances ranged from 0.14 to 0.20 nm from the protein surface (Table 2). Among solutes, Mg^{+2} , PO_4^{-2} , glucose, and CO_3^{-2} showed the most pronounced proximity to the SHBG surface. Comparing distance versus energy presented in Tables 2 and 3, an apparent contradiction in the results may be noted. Such behavior may be due to electrostatic screening effects promoted by clustering, such as ion–water and ion–ion interaction [63, 64]. The degree of electrostatic screening will modulate the energy magnitudes and not only the distance between salt-SHBG and salt-hormone, indeed. Furthermore, previous studies reveal that dielectric constant shows local fluctuations in the radial distance, credited to the ionic type and solute concentration [65, 66]. Thus, different levels of attractions and repulsion forces emerge and dynamics effects on the dielectric constant, mediated by the different ions and concentrations, may contribute to the obtained values observed in Tables 2 and 3. Nevertheless, we recognize the importance of future investigations for better quantifying these effects in the aqueous solution assumed in this work.

Solute assembly was already reported in solutions composed by sodium iodide dissolved in ether solution and aqueous NaCl solutions at high pressures and temperatures [67–70]. This result suggests the existence of a detainment state, already emphasized by previous investigations carried out on the ion-residues system [71]. Furthermore, since the simulations were run during a certain period, this detainment might constitute an intermediary or temporary stage before glucose finally is able to form a covalent bond with SHBG molecule.

The present calculations revealed persistent instabilities in RMSD pattern as well. However, this represents a plausible biophysical scenario if one considers a complex fluid composed by different constituents. In other words, time required for the system stabilization, quantified by the RMSD analysis, depends on many factors as, for example, the ionic species involved [72]. Indeed previous reports have shown that instabilities seems to be present in other systems,

Table 3 Electrostatic and Lennard-Jones interactions between E2 and aqueous solution components

Energy (kJ/mol)	Ca ²⁺	Cl ⁻	K ⁺	Mg ²⁺	Na ⁺	CO ₃ ²⁻	PO ₄ ²⁻	Glucose
Coulomb (SR)	(6.2 ± 0.2), 10 ⁻⁸	(0.84 ± 0.02), 10 ⁻⁴	(5.1 ± 0.1), 10 ⁻⁴	(2.4 ± 0.1), 10 ⁻⁸	(- 6.1 ± 0.3), 10 ⁻⁵	(- 1.2 ± 0.0), 10 ⁻²	(- 4.89 ± 0.04), 10 ⁻⁶	(- 2.69 ± 0.02), 10 ⁻²
LJ (SR)	(- 8.5 ± 0.2), 10 ⁻⁸	(- 0.38 ± 0.1), 10 ⁻⁴	(- 0.26 ± 0.01), 10 ⁻⁴	(- 3.3 ± 0.2), 10 ⁻⁹	(- 0.21 ± 0.09), 10 ⁻⁵	(- 0.1 ± 0.0), 10 ⁻²	(- 0.25 ± 0.22), 10 ⁻⁶	(- 0.52 ± 0.03), 10 ⁻²
Distance (nm)	1.20 ± 0.00	0.99 ± 0.00	1.00 ± 0.00	1.20 ± 0.00	0.21 ± 0.00	0.73 ± 0.00	1.12 ± 0.00	0.49 ± 0.00

Standard deviations were omitted due to their very small values

Fig. 7 Interactions between E2 and SHBG created with Maestro software

independent of simulation time (ns): 4,10,50,130,400, and 1000 [38, 73–78]. Thus, based on Chen et al., one similarly suggests that SHBG+E2 immersed in solution with different ions/concentrations may promote fluctuations in the RMSD profile [79]. SHBG fluctuation arises as a mechanism for binding of ligands, which could facilitate E2 releasing on the receptor site. Additionally, influenced by an interpretation given by Gao et al., fluctuations in the RMSD might be due to ligands continuously trying to achieve an appropriate position to maintain the interactions within the protein [77].

The glucose cluster may be characterized by the formation of more hydrogen bonds (or saline bonds) or due to local density increment followed by preferential orientation of glucose molecules in contrast to its ambient. In this sense, the high energetic values for glucose are explained by the partial water extinction from regions close to the SHBG with consequent saline bridges or hydrogen bonds formation ruling solute interactions [78–80]. From a physiological perspective, glucose clustering near SHBG may emerge as a beneficial mechanism for controlling the glucose freely available in the plasma associating SHBG with type 2 diabetes [23]. Indeed, although their molecular mechanisms remain unknown, genetic evidence suggests that lower SHBG levels are observed in type 2 diabetes patients as compared to non-diabetic individuals. Glucose assembly may also regulate protein conformational stability in the response of external perturbations [81–83]. In fact, Ohan and Dunn showed that glucose is a useful additive to stabilize collagen sterilized with irradiation while Arakawa and Timasheff associated the area increment of different proteins, promoted by glucose, as the main factor responsible for a stable state [82–85]. This argument is congruent with results obtained by Lins et al., considering interactions between trehalose with lysozyme protein in

Table 4 Average of the binding energy components for E2

Energy (kJ/mol)	V_{vdw} (bond)	V_{vdw} (free)	V_{el} (bond)	V_{el} (free)	ΔG
E2	-146.52 ± 0.01	-12.09 ± 0.01	-71.75 ± 0.01	-26.87 ± 0.02	-54.68 ± 0.01

Results are expressed as mean $\pm$ standard deviation

water solution at room temperature [86]. According to their results, trehalose moves toward protein by crowding around the lysozyme surface due to water molecules partially removed away from the protein surface. Based on their findings, clustering was interpreted as an important element to promote lysozyme stabilization as well. In this context, glucose self-assembly arises as general mechanism for conformational stability enhancement of globular proteins against temperature changes [51]. However, deleterious effects of high concentrations of glucose and consequent crowding on SHBG are not discarded. This observation is influenced by previous work carried out with macromolecular aggregates composed by Dextran 70 and anionic protein lysate from *Escherichia coli* [87]. This hypothesis could be assessed using glucose concentrations from the normal to the diabetic range. From these observations, more investigation on the glucose concentration influence on SHBG is necessary to determine the effects of such assemblies.

The present work provided an asymmetric and non-uniform density for RDF, considering water and ions around E2. This curve shape is similar to results obtained in previous investigations [88]. It is important to highlight that an analogous RDF pattern was also documented in aqueous NaCl solution simulations [89]. Considering other systems, RDF also exhibits an asymmetric curve, as attested to studies carried out by Filipponi et al. [90]. These authors explained the RDF asymmetry in terms of unharmonic lattice vibrations, comparing experimental data and theoretical calculations. In the present work, one suggests asymmetric and non-uniform density for RDF as combination of distinct salt concentration and interaction energy between ions and E2. This interpretation is strengthened by molecular dynamics simulation of electrolyte solutions, revealing asymmetric and non-uniform density for RDF [91].

Since E2 is partially soluble in aqueous solution, an important issue consists in identifying which solute component interacts more intensely with this hormone. In this framework, a theoretical inspection becomes especially relevant to understand the free but biologically active E2 in serum, where the hormone is exposed to the aqueous fluid while it moves toward a binding site. In the present report, an energetic analysis reveals that glucose is the main solute component interacting with E2: $(-2.69 \pm 0.02) \cdot 10^{-2}$ kJ/mol and $(-0.52 \pm 0.03) \cdot 10^{-2}$ kJ/mol for Coulomb and LJ, respectively. Based on this calculation, it is possible to conclude that hormone diffusion within the aqueous solution was mainly influenced by attractive forces acting on E2–glucose interaction. It was also hypothesized if the aqueous medium interferes with the structural dynamics of SHBG, perturbing E2 binding with the substrate. The results gave a $\Delta G = -54.68 \pm 0.01$ kJ/mol for SHBG-E2 complex, while other authors found $\Delta G = -48.9$ kJ/mol in experiments done in the human plasma [51]. Such difference in this result is related to the specific methodological procedure adopted in comparison with previous studies. Last, SASA calculations showed that clustering promotes increment of the surface SHBG area without affecting ligand or protein binding site areas. This result is supported by the Ben-Naim hypothesis, which proposes that local modifications in protein folding, derived by the local peptides interactions, do not mean modifications in other areas of the protein [57]. From the experimental point of view, the calculations also converge to experimental investigations showing that carbohydrate interactions with SHBG do not affect steroids bind properties [92]. In addition, the affinity result obtained provides the magnitude of hormones releasing from the active site of SHBG to the estrogen receptor. Nevertheless, since this finding is based on simulations, experimental determination of ΔG is necessary to reinforce more accurately our theoretical values.

In summary, to the best of our knowledge, this is the first report to combine MD and molecular docking for investigating a complex aqueous environment, which mimics the salts found in physiological solution constituents. In the future, other relevant issues would consist of examining glucose cluster regulation by ionic concentration, pH, and temperature changes. In addition, to achieve a more realistic physiological scope, studies should consider salt concentrations completely compatible with a Ringer solution. Finally, further work is required to determine specific SHBG residue sites or attractor domains interacting with glucose and ions.

4 Conclusions

The present work contributes to a better understanding of energetic aspects related to the E2–SHBG–salt interaction in a complex fluidic environment. The calculations characterized the energetic aspects of the electrolyte solution interaction with SHBG and E2, highlighting a remarkable interaction energy between glucose and SHBG surface. Surprisingly, movement of solute components toward SHBG promoted clusterization surrounding the protein. However, clustering increased the surface SHBG area without affecting ligand or protein binding site areas. Simulations also revealed an interaction between E2 and glucose, considering the hormone immersed in the solution.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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