How does fatty acid influence anti-thyroid drugs binding and specificity toward protein human serum albumin? A blind docking simulation study

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Abstract. This study reports an AutoDock-based blind docking simulation investigation to characterize the binding interaction of a series of anti-thyroid drugs (2-mercapto-1-methylimidazole (MMI), 2-thiouracil (TU), 6-methyl-2-thiouracil (MTU), 6-*n*-propyl-2-thiouracil (PTU) with a model plasma protein Human Serum Albumin (HSA) in the presence and absence of fatty acid (FA). The drug-protein binding efficiency is characterized in terms of binding free energy and the association constant (K_a , which is estimated as the reciprocal of the inhibition constant, K_i) of the drugs to the transport protein. The study also unveils the substantial impact of the presence of fatty acid (FA) on the binding interaction process. It is shown that in the presence of FA the drug-protein binding efficiency is markedly enhanced (except for MTU) and the binding location is changed. Hydrogen bonding interaction appears to play a governing role in the process of FA-induced modifications of binding efficiency and location.

Keywords. Anti-thyroid drugs; human serum albumin; fatty acid; binding specificity; blind docking simulation.

1. Introduction

Human serum albumin (HSA), the most abundant human plasma protein, contains a single polypeptide chain of 585 amino acids.¹ It is largely helical and composed of three structurally homologous domains (I, II and III). The structural organization of HSA provides several ligand binding sites. HSA displays seven binding clefts hosting chemically diverse ligands including fatty acids (FAs) and are labelled as FA1–FA7 (scheme 1). FA3 and FA4 comprise the Sudlow's site II (in sub-domain IIIA) and FA7 represents Sudlow's site I (in sub-domain IIA). As the major soluble protein constituent of the circulatory system, HSA has many physiological and pharmacological functions. It contributes to colloid osmotic blood pressure and is chiefly responsible for maintenance of blood pH.^{1,2} HSA plays an instrumental role in the transport and disposition of many exogeneous and endogeneous compounds. Its surprising capacity of binding to a large variety of drugs results in its prevailing role in drug pharmacokinetics and pharmacodynamics.^{1,2} It may also lead to interference with the binding of other ligands as a result of overlap of binding sites and/or conformational modifications.^{3–5} The protein can also lead to metabolic

modification of some ligands and/or alteration in pharmacokinetic behaviour of drugs by holding them in strained configuration. Detailed knowledge of the drug-albumin interaction is therefore essential in order to achieve in-depth understanding of the pharmacokinetic behaviour of the drug and is also important to capitalize on the probabilities of design of analogues with more favourable pharmacological properties.

In the present program we report a molecular docking simulation investigation on the interaction of anti-thyroid drugs 2-mercapto-1-methylimidazole (MMI), 2-thiouracil (TU), 6-methyl-2-thiouracil (MTU), 6-*n*-propyl-2-thiouracil (PTU) (scheme 1) with model plasma protein HSA (defatted) and fatty acid bound-HSA. The drugs (MMI, TU, MTU and PTU) are known for traditional treatments of Grave's disease.^{6,7} These drugs are known to function by depressing the formation of the thyroid hormones, triiodothyronine and thyroxine. Anti-thyroid drugs are rapidly and almost completely absorbed after oral administration and concentrated in the thyroid gland.⁷ Our results also highlight that the presence of fatty acids can lead to discernible modifications in anti-thyroid drug-HSA binding interaction in terms of binding location, nature and efficiency of binding. This appears to be relevant in rationalizing the interference between dietary compounds, food supplements and drugs.

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Scheme 1. Cartoon representation for the (a) structure of defatted HSA (PDB entry: 1AO6) and HSA-fatty acid (FA) complex (PDB entry: 1GNI) with labeling of the FA binding sites represented as Corey-Pauling-Koltun (CPK) model in red. TRP-214 of HSA is shown as CPK model in blue. (b) Chemical structures of the drugs investigated.

Among the four aspects of pharmacokinetics (absorption, distribution, metabolism and excretion), distribution is the one that is controlled by serum protein because most drugs that travel in plasma bind to protein.^{1,2} In other words, the degree of bioavailability of a drug will, to a large extent, depend on the free concentration of the drug in plasma. Thus, factors that may affect albumin binding properties form a central issue in pharmacology. An important question is whether the binding of physiological substances or other drugs can influence the interaction of albumin with a given drug. The levels of free fatty acids in plasma are known to vary considerably.⁸ FA is known to influence the drug binding properties of albumin allosterically^{8,9} and thus underlining the role of FA in modulating ligand binding to HSA. As free fatty acids bind primarily to albumin,⁸ it would be of particular interest to study how the binding properties of drugs toward albumin are modified due to the presence of fatty acid.

2. Method

The native structure of HSA was taken from the Protein Data Bank with PDB ID: 1AO6.¹⁰ Docking studies were performed with AutoDock 4.2 suite of programs which utilizes the Lamarckian Genetic Algorithm (LGA) implemented therein. For docking of drugs with HSA, the required file (corresponding to the three-dimensional structure of drugs) for the ligand (drug) was created through combined use of Gaussian 03W¹¹ and AutoDock 4.2^{12,13} software packages. The geometry of the drugs was first optimized at DFT//B3LYP/6-31+G(d,p) level of theory using Gaussian 03W suite of programs and the resultant geometry was read in AutoDock 4.2 software in compatible file format, from which the required file was generated in AutoDock 4.2. The grid size was set to 126, 126, and 126 along X-, Y-, and Z-axis with 0.408 Å grid spacing, i.e., in order to recognize the binding site of the drugs in HSA blind docking was performed. The AutoDocking parameters

used were as follows: GA population size = 150; maximum number of energy evaluations = 250000; GA crossover mode = two points. The lowest binding energy conformer was searched out of 10 different conformers for each docking simulation and the resultant one was used for further analysis. Similarly, for docking study with HSA with bound oleate molecules, the atomic coordinates were taken from PDB entry 1GNI.¹⁴ The PyMOL software package was used for visualization of the docked conformations.¹⁵

3. Results and Discussion

3.1 Drug-protein (HSA) binding interaction

In recent times, *in silico* molecular docking technique has garnered considerable attention in various fields of biophysical and biochemical research including structure-based drug design. Most applications of docking tools focus on the primary binding region. However, a major bottleneck of this approach is that

specific information on the binding region is often missing. The AutoDock¹² based blind docking approach has been successfully exploited to overcome this limitation and evaluate unbiased results on the probable interaction site of drugs in the protein.^{13,16} The blind docking approach includes a search over the entire surface of proteins for binding sites while simultaneously optimizing the conformations of the peptides^{13,17} whereby indulging in an unbiased result, and hence has rightfully been described as “very encouraging” in a recent review.¹⁷ Herein, we have emphasized on the application of blind docking strategy to explore the binding interaction of the drugs MMI, TU, MTU and PTU with HSA.

Figure 1 illustrates the stereo view of the minimum energy docked conformations of the drugs with HSA in which the binding sites are indicted within a solid circle. It is evident from the figure that the AutoDock-based blind docking simulation predicts hydrophobic sub-domain IIA as the favourable binding site for the drugs MMI and TU (with MMI being more embedded into the sub-domain IIA while TU is rather closer to

Figure 1. View of the docked conformation of the antithyroid drugs (a) MMI, (b) TU, (c) MTU, (d) PTU with the protein HSA. The site of interaction of the drugs is specified in red circle. The drugs (blue) are represented in Corey-Pauling-Koltun (CPK) model. The pictures have been prepared on PyMOL software package.¹⁵

the interface of sub-domains IIA and IIB), while PTU is located in sub-domain IB and for MTU the predicted favourable binding site is found to be at the interface of sub-domains IB and IIA. These findings are also found to bear reasonable consistency with reported literature.^{18,19} The favourable binding interaction of all the drugs with the protein is predicted from negative binding free energy (BE) (table 1. Also, see Supplementary Information for the energy decomposition analysis of the estimated free energy of binding). It is seen that the most favourable binding of HSA occurs with drug MTU (BE = −3.49 kcal/mol) with comparable BE of PTU (−3.23 kcal/mol). The data summarized in table 1 reflect a discernible harmony of the drug-protein association constant, K_a values with the BE values. Figure 2 shows an enlarged view of the minimum energy docked conformations at the sites of interaction of the drugs marking the nearby protein residues, which is also enlisted in table 1. A hydrogen bonding interaction is detected for interaction of PTU with ARG-141 (the hydrogen bond

is formed between the carbonyl oxygen of ARG-141 and the ring N–H moiety of PTU. The H-bond distance is = 2.086 Å, θ (donor–H–acceptor angle) = 127.14°, H-bond energy = −0.67 kcal/mol (the parameters are as obtained from the minimum energy docked conformation). No H-bonding interaction with nearby protein residues is detected for the rest of the three drug molecules (figure 2). Figure 2a reveals that the orientation of the drug MMI within the protein cavity is such that it is almost parallel to the nearby tryptophan residue (TRP-214) of the protein. The short distance of separation (4–5 Å, as obtained from the minimum energy docked conformation) between the drug (MMI) and the TRP-214 residue is noted. It is not unlikely to assume the operation of interaction of the lone pair and/or π -electron cloud of the drug (MMI) with the π -electron cloud of TRP-214 leading to perturbations in the TRP microenvironment. Cowburn *et al.*²⁰ calculated the rotational strength for such an inter-chromophoric interaction and showed that it is critically dependent upon the relative orientation

Table 1. Summary of the AutoDock-based blind docking simulation results for interaction of the drugs (MMI, TU, MTU and PTU) with fatty acid free HSA and HSA in the presence of fatty acids (T = 298.15 K).

Drug	Binding free energy (kcal/mol)	Association constant, K_a ($10^2 \times \text{mM}^{-1}$)	Protein residues within 5.0 Å of the interaction site of the drug ^a
Docking with HSA (PDB entry: 1AO6)			
MMI	− 1.97	2.76	LYS-195, LEU-194, TRP-214, PHE-207, SER-198, LEU-477, LEU-343
TU	− 2.53	7.12	LEU-194, TRP-214, SER-450, VAL-340, LEU-477, PHE-207, VAL-478, ALA-206, LEU-343
MTU	− 3.49	36.5	GLU-184, HIS-284, PHE-153, GLU-288, SER-188, GLU-149, ALA-187, ALA-287, LYS-191, TYR-146, ARG-253
PTU	− 3.23	23.2	PRO-106, ARG-141 (2.086 Å) , HIS-142, PHE-145, TYR-144, ARG-193, PRO-143, ALA-190, GLN-455, ASP-104
Docking with HSA-FA (PDB entry: 1GNI)			
MMI	− 3.09	18.32	GLU-450, SER-454, LEU-481, VAL-482, ARG-484, PRO-486, ARG-348, ARG-485, FA-3 (2.183 Å)
TU	− 3.43	32.89	LEU-453, LEU-457, SER-454, ARG-484, LEU-481 (1.853 Å) , VAL-482, ARG-348, ASN-483, VAL-344, PRO-480, ARG-485, FA-3
MTU	− 3.47	35.21	LEU-453, SER-454, LEU-457, ARG-484, LEU-481, VAL-432, ASN-483, ARG-348, PRO-486, ARG-485, FA-3
PTU	− 4.06	95.24	LEU-453, SER-454, ASN-458, LEU-198, LEU-457, GLU-450, ARG-484 (2.099 Å) , SER-202, LEU-481, TRP-214, VAL-344, ARG-485, FA-3

^aThe residues indicated in bold fonts are involved in H-bonding interaction with the respective drug molecules. The H-bond distance is shown in parenthesis.

Notes: LYS = Lysine; LEU = Leucine; TRP = Tryptophan; TYR = Tyrosine; PHE = Phenylalanine; SER = Serine; ALA = Alanine; VAL = Valine; HIS = Histidine; GLU = Glutamic acid; GLN = Glutamine; PRO = Proline; ASN = Asparagine; ASP = Aspartic acid; ARG = Arginine; MMI = 2-mercapto-1-methylimidazole; TU = 2-thiouracil; MTU = 6-methyl-2-thiouracil; PTU = 6-n-propyl-2-thiouracil; HSA = Human Serum Albumin; FA = Fatty acid.

Figure 2. Protein residues in near vicinity of the drugs: (a) MMI, (b) TU, (c) MTU, (d) PTU, over a molecular surface representation of the protein. Colour scheme: white for hydrogen atoms, blue for nitrogen atoms, red for oxygen atoms and green for carbon atoms. Hydrogen bonding interaction is shown in yellow dotted linkage. The pictures have been prepared on PyMOL software package.¹⁵

of the transition moments. The circular dichroism (CD) spectroscopy can reflect some glimpse of such interactions.^{20–22} Since the transitions are $\pi-\pi^*$, the transition moments are all in-plane and the excitonic or the off-resonance contributions, or both, might be reduced upon substrate binding, which should be manifested in a decrease in the CD signal of the protein upon MMI binding. A recent report from our laboratory has shown observations in consensus with this line of arguments.²¹ The interaction of the drugs TU and MTU seems to be mainly governed by hydrophobic forces.

Effect of fatty acid: The overall interaction pattern of the drugs is found to be enormously modified in the presence of FA. Figure 3 unveils that in the presence of FA the favourable binding location of all the drugs investigated is the hydrophobic sub-domain IIIA (Sudlow's site II). It is seen from the minimum energy docked conformations (figure 3) that the drugs MMI and MTU are more embedded into the hydrophobic pocket of the protein sub-domain IIIA while TU and PTU are relatively less buried into the pocket with PTU being more exposed to the outer surface. The data

compiled in table 1 demonstrate that the presence of FA renders the drug-protein interaction much more favoured than with fatty acid free HSA as evident from the binding energies obtained from automated blind docking simulation (table 1). The values of association constant (K_a) are also found to reflect a similar trend. The drug binding affinity with protein is found to be remarkably enhanced in the presence of FA, e.g., ~ 6.64 times for MMI, ~ 4.62 times for TU and ~ 4 times for PTU. At the same time, it is worth noting that the association constant (K_a) for binding interaction of the drug MTU with the protein is not significantly modified in the presence of FA (rather the presence FA results in slight decrease in K_a , table 1). Such differential behaviours of the drugs can be rationalized on the basis of their ability to form hydrogen bond with the nearby protein residues/FA residue at the respective sites of interaction within the protein cavity. The drug MMI is found to form a H-bond with FA3 (the H-bond formed between the ring N–H moiety of MMI with the oxygen atom of FA3, H-bond distance = 2.132 Å, θ (donor–H–acceptor angle) = 159°, H-bond energy

Figure 3. View of the docked conformation of the anti-thyroid drugs (a) MMI, (b) TU, (c) MTU, (d) PTU with the protein HSA after fatty acid binding. The site of interaction of the drugs is specified in blue circle. The drugs (blue) and fatty acids (red) are represented in Corey-Pauling-Koltun (CPK) model. The pictures have been prepared on PyMOL software package.¹⁵

= -4.25 kcal/mol), the drug TU is found to form a H-bond with LEU-481 (the H-bond formed between the ring N–H moiety of TU with the carbonyl oxygen of LEU-481, H-bond distance = 1.85 Å, θ (donor–H–acceptor angle) = 154.57° , H-bond energy = -5.28 kcal/mol), the drug PTU is found to form a H-bond with ARG-484 (the H-bond formed between the carbonyl oxygen of PTU with the N–H moiety of ARG-484, H-bond distance = 2.099 Å, θ (donor–H–acceptor angle) = 172° , H-bond energy = -5.21 kcal/mol). The blind docking simulation results thus predict the formation of considerably strong H-bonds of the drugs MMI, TU and PTU with nearby protein residues/FA residue. Such additional stabilization through H-bonding interaction is likely to contribute to the driving force for a more favoured interaction of the drugs with HSA in the presence of FA in comparison to that in fatty acid free HSA. The lack of such additional stabilization, as predicted from the blind docking simulation results, for MTU may be a factor responsible for the observed insignificant perturbation of the binding interaction of the drug with the protein in the presence of FA, i.e., even in the presence of FA the binding of MTU with HSA is principally governed by hydrophobic interactions.

Figure 4 yields a visual comparison of the possible interactions of the studied drug molecules with the nearby protein residues showing the H-bonding interactions for MMI, TU and PTU.^{23,24}

It is relevant to mention in this context that the docked poses of the drugs to the protein correspond to the best-energy solutions in the most populated cluster (representative figures are shown in figure 5).

3.2 Drug site in defatted HSA and HSA-FA complex

Problems associated with adsorption, distribution, metabolism and elimination (ADME) of drugs still pose considerable challenges to the field of development of new drugs^{22,25,29} and are hence driving the search for techniques to optimize ADME characteristics at an early stage in the design process. One of the most important factors affecting the distribution and the free, active concentration of many administered drugs is the binding affinity toward transport protein HSA. Many commonly used drugs bind to HSA, usually at one of two primary sites, designated as drug site 1 and drug site 2, located in subdomains IIA and IIIA, respectively.^{26,27} A certain degree of albumin-binding may be enviable

Figure 4. Protein residues in near vicinity of the drugs: (a) MMI, (b) TU, (c) MTU, (d) PTU, over a molecular surface representation of the protein upon binding with fatty acids. Colour scheme: white for hydrogen atoms, blue for nitrogen atoms, red for oxygen atoms and green for carbon atoms. Hydrogen bonding interactions are shown in yellow dotted linkages. The pictures have been prepared on PyMOL software package.¹⁵

in promoting solubilization of compounds that would otherwise aggregate and thus impairing a uniform distribution within the body. But drugs with an excessively high affinity toward protein require correspondingly higher doses to achieve the effective concentration *in vivo*. Also, a high binding affinity may impair the process of efficient distribution of the drug to the sites of action and their elimination.^{27,29} Structural information on HSA–drug interactions is thus very crucial and is receiving attention very recently, though the field still demands ample exploration.^{27,30–32} However, the interpretation of competition or binding data is not a trivial task given the identification of partially overlapping binding compartments in site 1,^{33,34} uncertainty as to the number of secondary drug binding sites on the protein^{34,35} and the possibility of allosteric interactions between drugs bound to sites 1 and 2.^{36,37} Further complexities emanate *in vivo* from interactions between drugs and endogenous ligands for HSA.^{37–39} This is particularly pertinent for fatty acids, which normally occur in serum at levels of between 0.1 and 2 mol/mol of HSA

and can both compete and cooperate with drugs binding to the protein. For certain disease states, these effects have been reported to be exacerbated with the rise of the fatty acid:HSA mole ratio to a value as high as six.^{31,39}

Thus, although correlations based on large datasets of measurements of drug binding affinity have highlighted the importance of molecular descriptors such as lipophilicity, acidity, hydrogen bonding potential and shape factors in determining albumin binding.^{30,35} Such ligand-based approaches are yet to provide a wholly robust method for predicting the affinities of new compounds, and structural information is clearly required to complement these investigations. The application of *in silico* approaches in structure-based drug design has been increasing in recent times not only because of cost-effectiveness of the technique but also due to the efficiency of performance.^{1,3–5,16,17,21,28}

Recently Curry *et al.* have reported crystallographic analyses to reveal the drug-binding specificity of HSA and the influence of fatty acids (FAs) involving different varieties of FAs.^{14,30} The binding of fatty

Figure 5. Representative examples for clustering analysis of docked poses for anti-thyroid drug binding to HSA in the absence and presence of fatty acid. RMSD used for the clustering = 2.0 Å.

acids, the primary physiological ligand for the protein, is shown to alter the polarity and increase the volume of drug site 1.^{14,29–31,35,40} In a recent report, Curry *et al.* have discussed the structural basis of the drug binding specificity of HSA including twelve drug molecules and the effect of FA-induced conformational changes was investigated with the structure of HSA-myristate complex.³¹ The authors have also reported that the results will also be applicable to more physiologically relevant FA e.g., oleate (as in the present case).³¹ Therefore, in the present program, we endeavour to explore the results obtained from Autodock-based blind docking simulation on the FA-induced conformational changes and its impact on the binding interaction of the anti-thyroid drugs in analogy to the crystallographic data as reported with the structure of HSA-myristate complex.³¹

Upon binding of fatty acids with HSA, TYR-150 from subdomain IB moves to interact with the carboxylate moiety of the lipid bound to the site that straddles domains I and II (fatty acid site FA2) (figure 6a and b).

This helps to drive the relative rotation of domains I and II and has a large impact on one side of drug site 1 (scheme 1). There is an extensive rearrangement of the H-bond network involving TYR-150, GLU-153, GLN-196, HIS-242, ARG-257 and HIS-288, which opens a solvent channel (between TYR-150 and GLN-196), thus increasing the volume of the pocket and altering its polarity distribution: the inner polar cluster is disrupted and partially neutralized by fatty acid binding; only HIS-242 is relatively unaffected (figures 6a and b). The helix containing LEU-198 is also displaced outwards. This appears to impact an adjacent helix from subdomain IIIA (residues 442 – 466) and its disulphide bonded neighbor. This latter helix is also twisted around its axis, since binding of FA3 in IIIA replaces GLU-450 in a salt-bridge interaction with ARG-348. As a result GLU-450 rotates to replace ASP-451 in interacting with the amide groups of residues 343–344. In turn, ASP-451 relocates to a position that allows it to form a salt-bridge with Lysine (LYS)-195 (figure 6c). This cascade of interactions indicates one possible link

Figure 6. Conformational changes in drug site 1 in HSA upon fatty acid binding. Close-up views of the region in the vicinity of TYR-150 in (a) HSA and (b) HSA-FA complex. (c) Rotated view of the region in the vicinity of GLU-450 and ASP-451 upon fatty acid binding. Both the residues GLU-450 and ASP-451 rotate to new positions upon FA binding: GLU-450 rotates to replace ASP-451 which in turn moves to form a salt bridge interaction with LYS-195. The pictures are prepared on PyMOL software package.¹⁵

between the two drug sites, at least in the presence of fatty acid.^{14,30,31}

Drug site 2 is composed of all six helices of subdomain IIIA and is therefore topologically similar to site 1 (subdomain IIA). Although, like site 1, it also comprises a largely pre-formed hydrophobic cavity with distinct polar features, there are significant differences between the two drug pockets. Drug site 2 is smaller than site 1; the principal binding region corresponds to the central portion of the site 1 pocket and appears to possess just one sub-compartment, the rear right-hand hydrophobic sub-chamber. To a large extent the left-hand sub-chamber is eliminated by the presence of TYR-411, which occurs in subdomain IIIA at the position corresponding to LEU-219 in IIA (figure 7a). A further difference arises because, although the two drug sites are in structurally similar subdomains, these are packed in different contexts with respect to the remainder of the protein. The entrance to drug site 1 is enclosed by subdomains IIB and IIIA; residues from these subdomains contribute to the formation of the front sub-chamber. However, the entrance to site 2 is not encumbered in this way: although IIIA is followed

by IIIB, this subdomain is rotated further away from the drug site entrance (in comparison to drug site 1, domain II) and leaves the pocket entrance more exposed to solvent (scheme 1). In contrast to drug site 1, site 2 has a single main polar patch, located close to one side of the entrance of the binding pocket and centred on TYR-411 but also including ARG-410, LYS-414 and SER-489 (figure 7a). Of these residues, only ARG-410 and LYS-414 occur in equivalent positions to polar-patch residues in drug site 1 (ARG-218 and ARG-222, respectively). Thus in terms of shape, size and polarity, drug sites 1 and 2 are clearly distinguishable and is thus not unlikely to contribute in the different binding specificities of the two pockets, viz., drug site 1 and drug site 2.

It is also noteworthy that although drug site 2 (subdomain IIIA) appears to be relatively small, it can bind two molecules of long-chain fatty acid (FA3 and FA4 in scheme 1).⁶ Comparison of drug and fatty acid binding reveals the very different ways in which these classes of ligand bind to a common site on the protein. In fact, drug site 2 is composed of the apolar region that is occupied by the methylene tails of FA3 and the polar

Figure 7. Drug site 2 in (a) defatted HSA and (b) HSA-FA complex. Binding of fatty acid leads to replacement of the GLU-450–ARG-348 salt bridge interaction in defatted HSA (panel a) with GLU-450–FA3 salt bridge interaction in HSA-FA complex (panel a). The pictures are prepared on PyMOL software package.¹⁵

patch that interacts with the carboxylate moiety of FA4. Moreover, FA3 does not interact with the polar patch centred on TYR-411. Instead, binding of the fatty acid opens access to a different polar patch by inducing the rotations to protein residues LEU-387 and LUE-453 and the lipid carboxylate group supplants GLU-450 in a salt bridge interaction with ARG-348 in subdomain IIB (figure 7b and c). These observations suggest possible ways in which drugs might be modified in order to take advantage of this flexible binding facility in the pocket. Fatty acid binding is also known to be associated with a large conformational change in HSA, involving rotations of domains I and III relative to domain II, which suggests a possible molecular mechanism for allosteric interactions between fatty acid binding sites.^{3–6,36,37} In contrast, the conformational changes observed for drug binding at sites 1 and 2 are more local; there is no evidence for the global conformational changes on the scale observed with fatty acid binding. The observed instances of allosteric interactions between drug sites 1 and 2^{35–37} may possibly be due to more subtle structural effects or to the presence of additional binding sites.

4. Conclusions

The present work demonstrates the Autodock-based blind docking simulation results on the interaction of a series of anti-thyroid drugs with the model transport plasma protein human serum albumin. The most interesting result of the study is that the presence of fatty acid (FA) exerts a profound influence on the drug-protein binding interaction process in terms of an altered locus of binding of the drugs on the protein backbone along with an enhanced binding efficiency, as compared with defatted HSA. The hydrogen bonding interaction appears to play a key role to promote the drug-protein binding strength. The results are also described in a consensus manner with the conformational change of the protein upon FA binding with particular focus on the two drug sites (1 and 2 located in subdomains IIA and IIIA, respectively).

It is known that HSA is an abundant plasma protein that binds to remarkably wide range of drugs and thereby restricting their free, active concentrations. Therefore, the problem of overcoming the binding affinity of drugs to HSA still poses a major challenge

in development of new drugs and thus also confronts the cost-effectiveness of the process. The modulations in drug binding affinity to HSA in the presence of fatty acids, the primary physiological ligand for the protein, is important in forming a rational template for design efforts to modulate the drug-HSA interaction. Furthermore, the considerable degree of harmony of our docking simulation results with reported crystallographic data substantiates the potential applicability of the *in silico* technique to the field, of course within an appreciable cost-effectiveness. We are optimistic that such *in silico* approaches to the field of structure-based drug design and investigation of the impact of other endogenous/exogenous ligands (e.g., physiological ligands like FAs) will achieve further important dimensions in near future.

Supplementary Information

The Supporting Information contains cluster analysis for anti-thyroid drug binding to HSA in the absence and presence of fatty acid, Energy decomposition analysis for the optimized docked conformations.

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