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Study of the interference effect of propranolol and amlodipine drugs on their interaction with human serum albumin based on molecular dynamics simulation method

M. R. Bozorgmehr[1*], M. R. Housaindokht[2,3]

[1] *Department of Chemistry, Mashhad Branch, Islamic Azad University, Mashhad, Iran*
[2] *Biophysical Chemistry Laboratory, Department of Chemistry, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran*
[3]*Institute of Biotechnology, Ferdowsi University of Mashhad., Mashhad, Iran*

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The interference effect of two drugs, namely propranolol and amlodipine on their interaction with human serum albumin (HSA) was investigated. For this purpose, HSA-bilirubin and HSA-warfarin simulation systems were used as a model for comparison with experimental evidences. Four simulations, i.e. "HSA only", "HSA-propranolol", "HSA-amlodipine" and "HSA-propranolol-amlodipine" were designed with equal proportions of drugs. A new algorithm was proposed in order to determine the residues of high affinity for binding to each drug. The new pattern is based on counting the times of drug referral to each residue during simulation. Binding sites of bilirubin and warfarin on the human serum albumin were determined in this manner; the residues in IIA and IIIA sub-domains assume high affinity for these drugs. The acquired results are in good agreement with experimental findings. To study the mutual drug effects, the binding sites of propranolol and amlodipine on protein were determined separately and in presence of both drugs. The obtained results indicated that in absence of drug interference, the number of residues having affinity for propranolol is larger than that for amlodipine; the number of residues having affinity to propranolol considerably decreases in amlodipine presence. The protein secondary and tertiary structure changes were compared in presence and absence of drugs for interpreting the obtained results.

Keywords: drug interaction, ligand binding, warfarin, tertiary structure

INTRODUCTION

Human serum albumin is the most abundant protein in blood plasma constituting 60% of the total blood protein. In adults' blood, albumin concentration is approximately 40 mg/ml [1]. It is known that this protein mobilizes different compounds including biliary salts, fatty acids, variety of drugs such as diazepam, warfarin and tamoxifen. The main physiological role of human serum albumin seems to be the transfer of metabolites and other soluble substances to target tissues through blood circulation, and stabilization of pH and osmotic pressure of blood plasma.

HSA is a single chain protein of 585 residues. This protein is composed of 3 homologous domains each of which is divided into 2 sub-domains. Domain I includes residues 1 to 195, domain II – residues 196 to 383 and domain III – residues 384 to 585. Each domain consists of ten helices; the first six helices form the sub-domain A and the latter four helices – the sub-domain B of each domain. 17 disulfide bridges and a free cysteine (CYS34) exist in the structure of the protein. Domains I, II and III have 5, 6 and 6 disulfide bridges, respectively. CYS34 residue is situated on the protein surface while its sulfur atom is located in the protein and is enclosed by the residues PRO35, HIS35, VAL77 and TYR84, preventing sulfhydryl groups from coupling with outer parts.

Analysis of the crystallographic structure of HSA indicated that there exist two major binding sites in the sub-domains IIA and IIIA for different compounds; they are designated as "binding site I" and "binding site II", respectively [2]. In most cases, hydrophobic residues form a hydrophobic hole playing a crucial role in drug mobilization. Furthermore, it is proved that fatty acids having large residues are bound to sub-domains IB and IIIB [3]. Warfarin binds to "site I" with high affinity. Some studies have shown that warfarin shares with drugs like amlodipine, aspirin and indometacin binding to "site I" [4]. Other investigations have indicated that low amounts of fatty acids or an increase in pH would lead to enhancement of warfarin binding affinity for HSA; however, its mechanism has not been well understood [2].

* To whom all correspondence should be sent:
E-mail: mr_bozorgmehr@yahoo.com

Beta blockers are those drugs used for controlling heart beats and heart protection after heart attack [5]. Propranolol is among such blockers. Amlodipine is a long-acting calcium channel blocker (dihydropyridine class) used as an anti-hypertensive agent and in the treatment of angina. The aim of this study is to evaluate the molecular dynamic simulation of the interference effect of these drugs in binding to HSA.

CALCULATION METHOD

All calculations were carried out by Gromacs software version 4.5.4 and the GROMOS 43a1 force field [6, 7]. It is demonstrated that the experimental observations of protein conformations and stability are reproduced by GROMOS force field in aqueous [8, 9] and non-aqueous [10, 11] media in high pressure systems [12] and at the interface of two different media [13]. Moreover, the comparison of GROMOS force field with other biomolecular force fields showed that GROMOS commonly delivers a better representation of the experimentally observed structural behavior of the proteins [14, 15]. Six simulation boxes in $8 \times 8 \times 8 \ nm^3$ dimensions were designed and HSA (PDB ID: 1AO6) was placed in the box centers. 100 molecules of warfarin, bilirubin, propranolol and amlodipine, respectively, were placed in each of the first four simulation boxes; 50 propranolol molecules and 50 amlodipine molecules were put inside the fifth box and no drug was added to the sixth one. Since drug potential parameters are not defined in Gromacs software, Dundee webserver was used to assign these parameters [16]. In order to use this web server optimized structure of the molecule of interest is needed. Optimized structures of propranolol, warfarin, bilirubin, and amlodipine were calculated using B3LYP/6-31G (d,p) *ab initio* methods implemented in Gaussian 03 quantum mechanics packages [17].Then all simulation boxes were filled with SPC water. For having neutral conditions in terms of electrical charge, suitable number of ions is added to each box.

To create a suitable starting point for simulation and eliminating the initial kinetic energy of the system, the energies of all abovementioned simulation boxes were minimized through steepest descent algorithm. Subsequently, all systems were simulated by position restrained algorithm; in this stage, the water molecules surrounding the protein were equilibrated. Then each grid was simulated for 50 nano-seconds with a 2 femto-seconds time-step. LINCS algorithm [18] was employed to fix the chemical bonds between the atoms of the protein and SETTLE algorithm [19] in the case of solvent molecules. All simulations were done at a temperature of 300 Kelvin. As simulation boxes contain molecules of different dimensions, each molecule will have its own specific kinetic energy; i.e. smaller molecules like those of the solvent have higher energy and larger molecules like protein have lower kinetic energy. Accordingly, the solvent will be warmer than the soluble matter during simulation. This is referred to as a "hot solvent-cold solute" phenomenon. In order to prevent this phenomenon and to control the temperature, each of the designed systems is coupled with a thermal bath. All boxes are coupled with a V-rescale bath to control system temperature. To calculate the electrostatic interactions, PME algorithm was used. In this algorithm every atom interacts with all atoms in the simulation box and all of their images in an infinite number of identical copies surrounding the main box, so that satisfactory results are produced for the electrostatic interactions [20, 21].

RESULTS AND DISCUSSION

Binding of drugs to a binding site is a dynamic process during which the drug moves in its binding site and can have different orientations. For determining the binding site of each drug, the following procedures were conducted:

a- The collisions between drugs and each of the protein residues are counted during simulation, n_i . For drug atoms being located at a distance not greater than 4 Å, each of the respective residue atoms is considered as a "collision". This distance is a limit proposed in references [22, 23].

b- Average numbers of collisions to each residue are computed. To calculate the average number, the total number of collisions is divided into a number of protein residues. $\langle n_i \rangle = {\sum n_i}/{n_r}$

In this relation, "n_r" is the number of protein residues (here 585).

c- Binding conformational factor is defined as $P_i = {n_i}/{\langle n_i \rangle}$. This quantity might assume different values signifying the following conditions:

The residue i with $P_i > 1$ is considered to have affinity toward the drug, while with $P_i < 1$ it has no affinity.

This simple algorithm was developed by our research group and was successfully utilized for predicting the binding site of thiourea to lysozyme,

the protein binding site of sodium dodecyl sulfate (SDS) to cobra cardio toxin, and the binding site of sodium dodecyl sulfate (SDS) to carbonic anhydrase [24]. The residues having P_i >1 in HSA-warfarin in the performed simulations were determined by this method and the results are listed in Table 1. According to the reported values, it is evident that in addition to residues in IIA and IIIA sub-domains which were reported as binding sites in the experimental reports [2], residues of domain I were also encountered. The results related to bilirubin are also reported in Table 1 and indicate that these residues are located in sub-domains IIA and IIIA, which is in agreement with experimental data. In Table 2, the residues having P_i >1 for HSA in presence of propranolol and amlodipine and equal proportions of each drug are presented.

Table 1 the residues having P_i greater than unit

HSA-Warfarin					HSA- bilirubin			
583	173	363	120	164	583	499	66	356
585	277	62	387	84	584	105	210	275
584	500	300	233	446	585	41	500	325
171	172	574	66	227	277	441	283	312
95	207	289	93	146	386	479	287	308
391	161	11	55	42	118	565	453	213
268	418	286	544	472	281	323	582	123
115	65	310	78	404	564	282	55	364
271	46	142	12	441	322	45	444	383
498	470	501	479		269	56	445	559
228	327	293	119		129	566	115	119
497	113	407	294		13	538	440	575
281	543	394	516		473	318	375	314
411	399	330	471		472	229	372	376
168	521	278	318		465	397	437	501
41	325	275	287		274	126	263	474
269	359	499	406		475	516	116	
145	206	110	582		443	399	373	
229	114	210	480		515	517	172	
398	550	547	303		390	497	305	
47	578	392	16		234	580	37	
157	540	130	283		398	514	449	
326	167	160	317		171	326	138	
270	468	77	61		496	360	468	
496	395	322	124		446	237	369	
267	560	514	517		12	44	206	
337	138	323	538		64	498	365	
112	34	135	230		495	65	396	
545	362	295	388		466	267	266	
117	546	410	74		570	280	324	
226	320	321	333		467	469	180	
282	290	412	118		574	5	230	
542	177	324	466		233	9	307	
469	127	116	51		521	379	168	
141	126	94	408		571	403	161	
541	559	174	575		359	476	387	
515	495	280	302		130	560	581	
319	403	301	69		418	87	270	
123	390	53	581		57	536	480	
64	213	50	360		117	6	58	

Table 2 the residues having P_i greater than unit

HSA- Amlodipine					HSA- Propranolol					HSA- Amlodipine-Propranolol				
85	578	233	92	512	585	375	545	389	496	319	583	479	310	549
584	495	358	439	383	581	318	546	435	571	573	584	399	418	89

580	41	298	317	582	463	322	468	70	467	585	205	369	313
579	225	80	295	583	539	532	520	273	480	398	138	85	500
583	45	52	300	580	362	356	323	241	118	83	238	403	108
581	557	96	575	584	577	359	253	294	296	61	498	81	317
582	244	249	471	579	76	308	81	324	98	322	546	362	94
125	353	373	173	260	94	574	361	306	109	480	302	296	575
277	364	94	48	167	465	78	171	314	124	492	467	177	16
264	360	505	555	559	578	125	56	188	262	497	312	577	271
263	53	552	266	393	392	299	286	169	338	59	319	543	367
393	538	309	246	263	201	570	477	345	115	496	472	135	90
260	462	78	487	562	558	390	326	557	534	268	581	574	517
497	301	90	483	369	226	414	466	320		476	339	364	300
556	553	122	467	576	386	90	209	377		270	60	363	303
394	392	49	62	365	372	295	126	101		320	121	289	15
390	310	55	100	9	379	80	168	316		368	120	209	297
91	539	366	461	560	73	267	82	398		473	324	326	522
558	352	491	89	203	8	493	77	575		550	582	330	494
369	576	315	267	57	206	278	358	490		161	13	468	481
46	559	493	313	264	266	298	563	550		207	469	545	578
501	494	335	414	277	86	475	92	561		82	323	208	37
318	484	469	305	60	497	46	337	426		542	490	397	63
387	506	283	163	83	55	495	127	130		318	62	470	127
230	38	7	241	6	491	376	166	41		580	376	274	579
496	365	8	463	116	469	185	87	489		269	180	58	371
126	504	34	276	476	108	394	311	104		206	64	366	11
468	492	273	226	373	205	131	274	291		299	301	293	372
319	465	76	472	225	321	366	58	222		267	365	134	204
560	54	79	362	5	265	229	492	395		105	391	488	573
265	577	81	279	230	164	13	461	110		495	257	477	493
499	314	278	435	202	567	464	59	285		394	298	547	14
308	73	574	64	382	91	256	544	61		314	519	260	373
561	297	124	51	30	102	360	364	512		84	80	518	420
511	361	510	498	227	79	129	52	297		241	501	359	168
299	44	564	74	237	439	97	508	240		157	327	551	499
167	201	164	480	114	54	184	472	380		321	264	181	395
502	229	391	128	315	566	74	543	53		12	122	86	
97	436	93	234	224	436	252	305	399		491	502	176	
359	562	30	127	442	462	353	335	276		338	277	315	

As indicated in the table, residues with affinity for propranolol drug exceed those affined for amlodipine in absence of drug interference. However, the numerical value of Pi for residues affined for amlodipine is larger than that of residues having affinity for propranolol (data not shown); thus, more alterations are expected in residues with affinity for binding to propranolol in the case of two drugs interference in binding to HSA. This happens because of the fact that amlodipine would primarily occupy the binding sites due to its higher affinity and both drugs will compete for binding to the protein. The residues with $P_i>1$ are illustrated in Table 2 for propranolol and amlodipine affinities in a system with equal proportions of both drugs. According to the table, it is seen that residues with $P_i>1$ remarkably decline in terms of propranolol affinity. Alterations in protein secondary and tertiary structural changes in the simulated systems were investigated for result interpretations. In Figure 1 the secondary structure of HSA along with its sequence is depicted to illustrate the numbers listed in Tables 1 and 2. In this figure red and blue colors show helices and coil secondary structures, respectively.

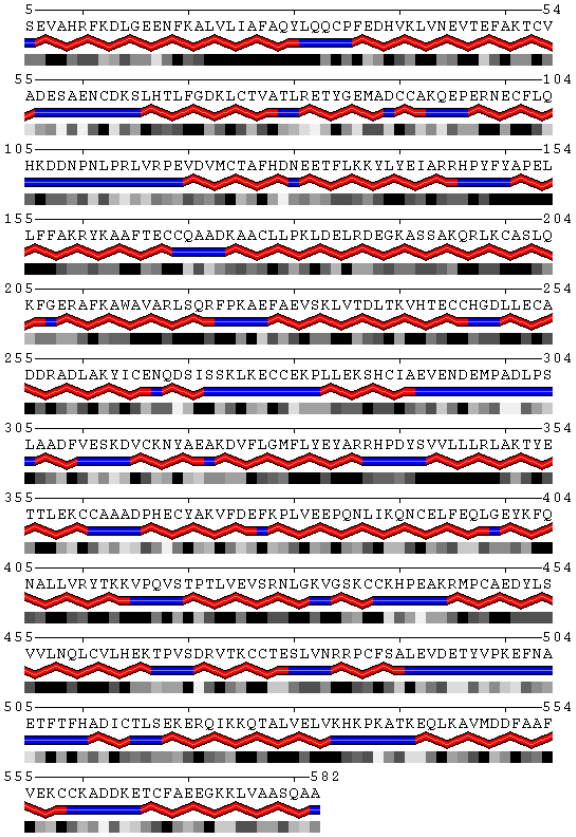

Fig. 1. Secondary structure of HSA along with its sequence depicted to illustrate the numbers listed in Tables 1 and 2.

Secondary Structure

Percentages of all structures including alpha helix, 3_{10} helix, turn and coil were calculated by the Kabsch-Sander method and DSSP program for HSA in the simulations [25]. The results are shown in Figure 2.

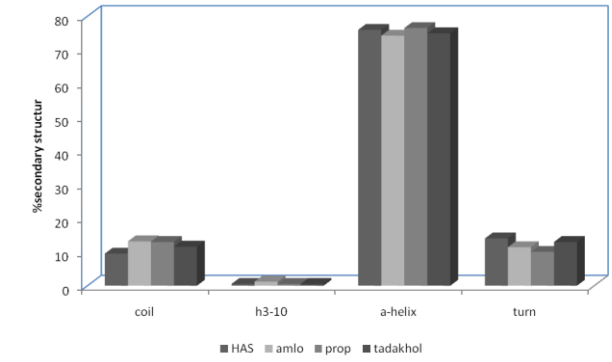

Fig. 2. HSA secondary structures percentage in the studied simulations.

As implied by the figure, amlodipine has a more reduced helix structure than propranolol, whereas the latter resulted in improved alpha helix structure to some extent. Turn structure has also decreased in the presence of propranolol and amlodipine. Turn and 3_{10} helix structures have increased in presence of amlodipine implying that in its presence alpha helix structure is converted into 3_{10} helix and coil structures. In general, the figure suggests that the protein secondary structural change is more considerable in presence of amlodipine compared to propranolol.

Tertiary Structure

Contact map was used to manifest the tertiary structure change of HSA in different conditions in this study. In Figure 3, the contact map of HSA is compared in presence and absence of propranolol.

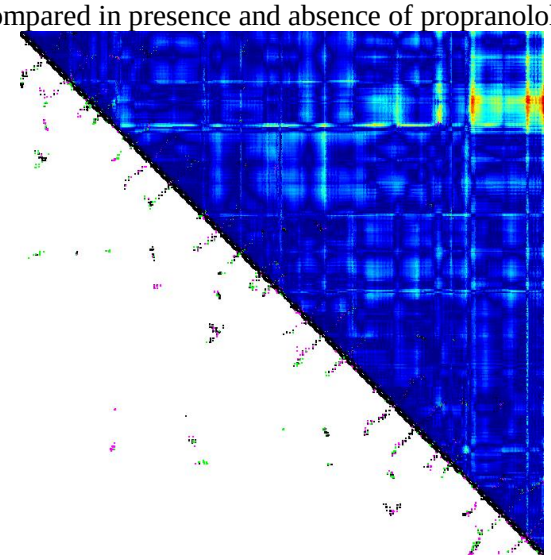

Fig. 3. Contact map of HSA in presence and absence of propranolol.

In the lower triangle of the contact map, pink color denotes the contacts existing in the protein in presence of propranolol but not existing in its absence, while green color represents the contacts existing in drug absence but not existing in its presence. Black color denotes the contacts in the HSA structure, which are common both in presence and absence of the drug. Variation curve of the distance between the i^{th} and j^{th} protein residues in presence and absence of drug is shown in the upper triangle of the contact diagram. Red and blue colors are related to the highest and the lowest changes of distance between the residues, respectively. According to the figure, the highest changes belong to the contact area of residues 66 and 495 and the contact region of residues 66 and 578.

In Figure 4, the contact map of HSA is compared in presence and absence of amlodipine. In the lower triangle of the contact diagram, green color specifies contacts existing in the protein in presence of amlodipine but not existing in absence of the drug, while pink color represents contacts existing in the protein in drug absence but not existing in its presence. Variation curve of the distance between the i^{th} and j^{th} protein residues in presence and absence of drug is shown in the upper triangle of the contact diagram. Extended variation

range is observed in the contact diagram compared to Figure 4 indicating that the protein tertiary structure is more influenced by amlodipine.

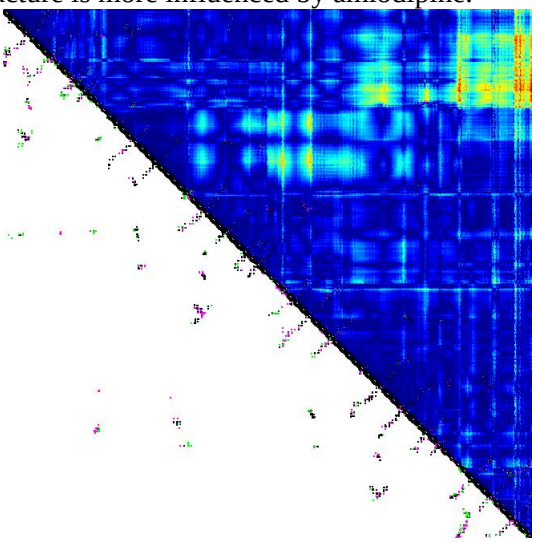

Fig. 4. Contact map of HSA in presence and absence of amlodipine.

In Figure 5, the protein contact map is compared in presence and absence of drug for equal proportions of both drugs.

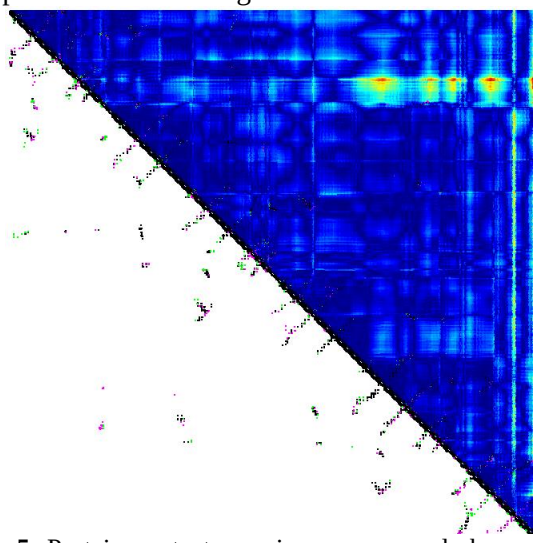

Fig. 5. Protein contact map in presence and absence of drug for equal proportions of both propranolol and amlodipine.

In the lower triangle of the contact diagram, green color signifies contacts existing in the protein in presence of amlodipine and propranolol but not existing in their absence, while pink color represents contacts existing in the protein in drugs absence but not existing in their presence. Needleman algorithm available in contact map view software was applied for generating the contact diagrams [26].

Hence, according to the abovementioned results, the protein structure is more influenced by amlodipine.

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ИЗСЛЕДВАНЕ НА ЕФЕКТА НА ИНТЕРФЕРЕНЦИЯ НА ПРОПАНОЛОЛ И АМЛОДИПИН ВЪРХУ ВЗАИМОДЕЙСТВИЕТО С АЛБУМИН ОТ ЧОВЕШКИ СЕРУМ ЧРЕЗ МЕТОДА НА СИНАМИЧНО СИМУЛИРАНЕ

М.Р. Бозоргмехр[1*], М.Р. Хусаиндокхт[2,3]

[1] Департамент по химия, Ислямски университет „Азад“, клон Машхад, Машхад, Иран
[2] Лаборатория по биофизична химия, Департамент по химия, Научен факултет, Университет „Фердоуси“в Машхад, Иран
[3]Институт по биотехнология, Университет „Фердоуси“ в Машхад, Иран

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Изследването има за цел да проучи ефектът на интерференция на две лекарства: пропанолол и амлодипин, върху взаимодействието с албумин от човешки серум (HSA). За тази цел са използвани системи за симулиране на HSA-билирубин и HSA-варфарин като модели за сравнение с опитни данни. Четири симулации, т.е. “само HSA”, “HSA-пропанолол”, “HSA-амлодипин” и “HSA- пропанолол - амлодипин” са конструирани с еднакви пропорции на лекарствата. Предложен е нов алгоритъм за определяне на остатъчните количества с висок афинитет на свързване за всяко лекарство. Предложен е нов модел на взаимодействие основан на преброяването на свързването на лекарствата към всеки остатък. По този начин са определени центровете за свързване на билирубина и варфарина върху HSA; остатъците в IIA и IIIA суб-домейни предполагат висок афинитет за тези лекарства. Получените резултати са в добро съгласие с опитните наблюдения. За изследването на съвместния ефект на лекарствата центровете на свързване на пропанолола и амлодипина върху протеините в присъствие на двете лекарства. Получените резултати показват, че в отсъствие на интерференция на лекарствата броят на остатъците с афинитет към пропанолола е по-голям от остатъците с афинитет към амлодипин. Броят на остатъците с афинитет към пропанолола намалява значително в присъствие на амлодипин. Измененията на вторичната и третичната структура са сравнени в писъствие и отсъствие на лекарства за интерпретиране на получените резултати.