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Investigation of Ethanol Effect on Albumin Active Site by Simulation Methods and Calculation of its Heat Capacity

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ABSTRACT

Serum Albumin is the most abundant protein in blood plasma. Its two major roles are maintaining osmotic pressure and depositing and transporting compounds. In this paper Albumin-Ethanol/Water solutions simulations are carried out by three techniques including Monte Carlo (MC), Molecular Dynamic (MD) and Langevin Dynamic (LD) simulations in aqueous solutions. Investigation the energy changes with time and temperature (between 273K to 303K) showed that MC technique is not suitable for macromolecule simulations. Also by comparing optimized energy in Albumin-water system and Albumin-Ethanol systems, it is distinguished that Albumin-Ethanol systems are more stable. So Ethanol can poison Albumin. Also the heat capacity of these systems is evaluated and it is shown that if the molecular mass of solvent increases, the value of heat capacity becomes more.

Keywords: Albumin; Simulation; Heat Capacity; Ethanol

INTRODUCTION

Albumin plays two important roles in body [1]: a) Transports some hormones and drugs. The most important compounds transported by Albumin are L-Tryptophan, Naproxen, Ibuprofen, Diazepam and fatty acids with medium chains [2]. b) Regulates osmotic pressure in body.

Sudlow has shown that Albumin has two active sites named Sudlow site I and II [2-4].Sudlow site II is more important and it can bind to ligands tightly by hydrophobic interactions [2]. It can bind and transport some important drugs such as those given above. Sudlow site I is less important than site II. It can transport drugs such as Warfarin, Salicylate and Sulphonamide. Its interactions are hydrophobic, too [2].

The understanding of the structural and thermodynamic properties of moderately or high concentrated solutions is fundamental, e.g., in medicine and biology and also in many technical processes[5-9].

Computer simulation of biological processes may serve several purposes. First, it provides a direct and precise way of checking an existing model. Secondly, it can be used to evaluate the relative importance of different parameters used in a model. Thirdly, and probably most important, attempts to construct computer models may help to improve or to sharpen the terminology of the concepts in question, because computer models can work only with precisely defined parameters[10-15].

Simulations can provide the ultimate detail concerning individual particle motions as a function of time. Thus, they can be used to address specific questions about the properties of a model system, often more easily than experiments on the actual system. For many aspects of biomolecular function, these details are

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of interest. Of course experiments play an essential role in validating the simulation methodology: comparison simulation between and experimental data serve to test the accuracy of the calculated results and to provide criteria for improving the methodology[16].

Because of the importance of Albumin as a drug transporter, in this paper Albumin simulation is carried out by three techniques including Monte Carlo, Molecular Dynamics and Langevin Dynamics and the effect of alcohol on its structure is investigated.

Monte Carlo (MC), Molecular Dynamics (MD) and Langevin Dynamics (LD) are three methods to simulate some molecules and macromolecules for understanding their structures and binding sites which can interact with other molecules[17].

COMPUTATIONAL METHOD

In this work, Albumin's active sites are downloaded from RCSB PROTEIN DATA BANK. The PDB ID applied in this paper is 1GAB in which the structure of Albumin-binding domain is investigated by NMR technique.

This PDB shows that active sites in chain A in Albumin consist of 53 residues (213-265) and its DSSP secondary structure is 73% helical (4 helices, 39 residues) and its chain type is polypeptide (L). Albumin's active sites sequences are as below:

THR ILE ASP GLN TRP LEU LEU LYS ASN ALA LYS GLU ASP ALA ILE ALA GLU LEU LYS LYS ALA GLY ILE THR SER ASP PHE TYR PHE ASN ALA ILE ASN LYS ALA LYS THR VAL GLU GLU VAL ASN ALA LEU LYS ASN GLU ILE LEU LYS ALA HIS ALA

After finding this PDB, HyperChem 7 software is applied for investigation in Ethanol and water solutions of protein separately.

At first these active sites are put in Water and Ethanol solutions which has concentration about 10% (w-w), separately. Then by using molecular mechanics level, opls force field and Polak-Ribiere algorithm, the geometry of these systems are optimized and for the optimized structures potential energy are evaluated by 3 simulation methods(MC, MD and LD) in different time steps and temperature range from 273K to 303K every 5 degrees. Then the potential energy versus temperature diagrams and potential energy versus time step diagrams are described in different initial temperatures in these force fields. Finally the energy of Albumin-water system has been optimized and compared by Albumin-Ethanol system.

It is considerable that because of the large gradient in energy, time steps are selected about 0.0001 for theses solutions in MD and LD techniques.

RESULT AND DISCUSSUIONS

The potential energy versus time step diagrams are shown in tables 1-6 and figures 1-3.

Table 1. Obtained potential energies in different time steps for Albumin-Water system in different initial temperatures calculated by MC method / ($kcalmol^{-1}$)

Time step	273 K	278K	283 K	288K	293K	298K	303K
0	-11413.2	-11413.2	-11413.2	-11413.2	-11413.2	-11413.2	-11413.2
10	-10692.4	-10671.7	-10652	-10644.9	-10608.2	-10614.8	-10590.8
20	-10492.1	-10519.9	-10446.2	-10468.9	-10446.5	-10383.2	-10337.9
30	-10387.9	-10434.8	-10402.3	-10343.9	-10373.5	-10296.9	-10252.6
40	-10323	-10328.4	-10270.1	-10244.2	-10270.2	-10283.9	-10181.9
50	-10302.5	-10271.7	-10195.1	-10231.8	-10222.4	-10198.8	-10181.6
60	-10270.8	-10230.9	-10211.6	-10217	-10196	-10152	-10156.5
70	-10258.2	-10236.7	-10144.3	-10163.9	-10128.8	-10128.3	-10123
80	-10194	-10204.9	-10169.5	-10132.8	-10119.3	-10113.3	-10043
90	-10196.9	-10201.1	-10079.1	-10086.3	-10072.4	-10071.9	-10065.2
100	-10148.5	-10141.1	-10104.4	-10093.9	-10073.5	-10003.9	-10039.2

Table 2. Obtained potential energies in different time steps for Albumin-Ethanol system in different initial temperatures calculated by MC method / ($kcalmol^{-1}$)

Time step	273K	278K	283K	288K	293K	298K	303K
0	-12008.9	-11846.7	-11992.7	-11899.5	-11553.8	-11284.3	-11577.7
10	-11216	-10991.2	-11130.7	-10995.2	-10646.3	-10378.6	-10661.5
20	-10966.2	-10758.3	-10882.2	-10740.7	-10413.9	-10133.9	-10391.3
30	-10867.3	-10601	-10782.7	-10689.2	-10305.6	-10018.4	-10285.4
40	-10818.4	-10584.9	-10716.6	-10602.7	-10205.3	-9964.83	-10202.4
50	-10736.7	-10541.9	-10728.1	-10531.1	-10211.2	-9937.63	-10165.5
60	-10718.9	-10478.1	-10650.6	-10524.4	-10149.6	-9873.72	-10124.5
70	-10667.5	-10443.7	-10577.3	-10494.2	-10131.2	-9818.62	-10137.6
80	-10667.4	-10437.4	-10529.5	-10436.9	-10068.3	-9851.46	-10091.5
90	-10635.5	-10406.8	-10495.3	-10386.7	-10072.3	-9781.26	-10050.2
100	-10582.9	-10369	-10500.8	-10393	-10057.1	-9724.44	-9988.45

Table 3. Obtained potential energies in different time steps for Albumin- Water system in different initial temperatures calculated by MD method / ($kcalmol^{-1}$)

Time step	273K	278K	283K	288K	293K	298K	303K
0	-11413.2	-11413.2	-11413.2	-11413.2	-11413.2	-11413.2	-11413.2
0.1	-10564.7	-10560.8	-10545.4	-10514.5	-10514.6	-10499.2	-10483.7
0.2	-10645.8	-10641	-10626.9	-10609	-10598	-10583.7	-10570.8
0.3	-10586.7	-10567.7	-10554.7	-10522.2	-10525.4	-10514.7	-10497.4
0.4	-10619.4	-10615.2	-10601.3	-10577.7	-10572.8	-10561.6	-10548.2
0.5	-10585.5	-10582.3	-10563.3	-10545.2	-10521.7	-10503.2	-10496.3
0.6	-10616.8	-10591.2	-10572.1	-10555.2	-10531.6	-10517.3	-10498.4
0.7	-10586.5	-10551.6	-10537.7	-10535	-10510.8	-10488.9	-10482.6
0.8	-10581	-10582.8	-10542.2	-10515.5	-10517.2	-10497.6	-10483.8
0.9	-10555.4	-10543.9	-10524.4	-10527.5	-10476.6	-10490.6	-10466.7
1	-10575.7	-10559.9	-10534.6	-10533.6	-10500	-10490.2	-10480.8

Table 4. Obtained potential energies in different time steps for Albumin-Ethanol system in different initial temperatures calculated by MD method / ($kcalmol^{-1}$)

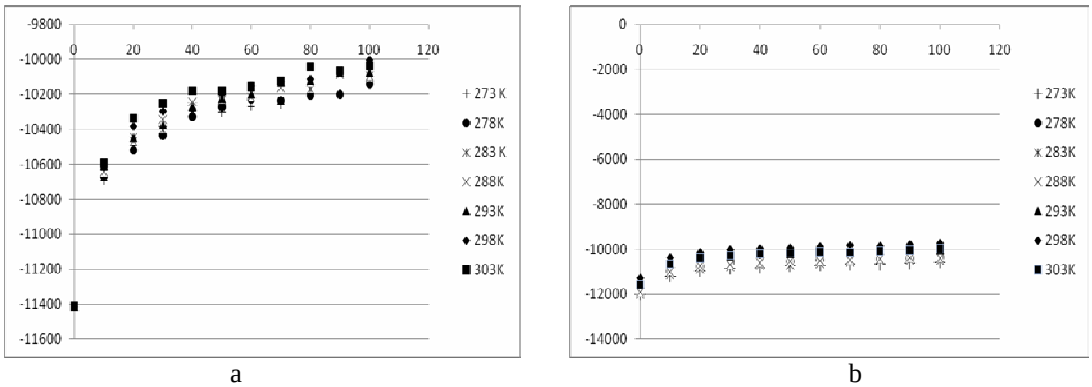
Time step	273K	278K	283K	288K	293K	298K	303K
0	-11497.6	-11555.1	-11404.7	-11542	-11530	-11627.7	-11411.4
0.1	-10566.6	-10595.2	-10424.1	-10540.9	-10543.3	-10591.3	-10407.8
0.2	-10637.4	-10695.2	-10495.1	-10649.3	-10640.1	-10716.3	-10472.7
0.3	-10617.1	-10636.2	-10471	-10576.2	-10562	-10599.2	-10435.5
0.4	-10655.1	-10700.2	-10501	-10627.7	-10602.9	-10668.5	-10482.1
0.5	-10597.7	-10628.8	-10461.2	-10611.7	-10572.2	-10617.2	-10412.8
0.6	-10628.6	-10653.4	-10497.1	-10636.3	-10592.5	-10647.2	-10438.2
0.7	-10638.5	-10649.2	-10477.3	-10599.3	-10563.6	-10610	-10433
0.8	-10623	-10607	-10467.3	-10584.6	-10584.4	-10611.3	-10426.9
0.9	-10607.1	-10631.5	-10459	-10585	-10586.2	-10590.5	-10391.9
1	-10626.3	-10617.5	-10487.9	-10571.6	-10548.5	-10598.7	-10441.1

Table 5. Obtained potential energies in different time steps for Albumin-Water system in different initial temperatures calculated by LD method / (kcalmol^{-1})

Time step	273K	278K	283K	288K	293K	298K	303K
0	-11413.2	-11413.2	-11413.2	-11413.2	-11413.2	-11413.2	-11413.2
0.1	-10562	-10559.1	-10545.4	-10514.5	-10514.6	-10467.8	-10483.7
0.2	-10654	-10649.3	-10626.9	-10609	-10604.1	-10609.2	-10570.8
0.3	-10571.7	-10573.7	-10554.7	-10522.2	-10535.5	-10533.1	-10497.4
0.4	-10628.1	-10618.5	-10601.3	-10577.7	-10580.1	-10559.6	-10547.9
0.5	-10579.7	-10584.1	-10563.3	-10545.2	-10536.5	-10503.7	-10495.8
0.6	-10620.5	-10585.5	-10572	-10555.5	-10555.8	-10522.2	-10499.1
0.7	-10575.8	-10586.3	-10536.4	-10534.6	-10536.6	-10520.2	-10481.3
0.8	-10576.9	-10582	-10537.2	-10524.3	-10527.6	-10484.9	-10489.3
0.9	-10584.5	-10571.2	-10515.6	-10526.6	-10546.8	-10513.1	-10461.8
1	-10561.2	-10562.4	-10529.1	-10530.4	-10521	-10514	-10476.4

Table 6. Obtained potential energies in different time steps for Albumin-Ethanol system in different initial temperatures calculated by LD method / (kcalmol^{-1})

Time step	273K	278K	283K	288K	293K	298K	303K
0	-11493	-11644.7	-11754.9	-11942.2	-11517	-11626.2	-11379.4
0.1	-10536.7	-10681.6	-10779.8	-10953.9	-10523.1	-10611.1	-10349.2
0.2	-10626.8	-10785.3	-10873.3	-11047.8	-10624.3	-10682	-10428.1
0.3	-10577.2	-10712	-10792.5	-10977.7	-10548.9	-10630.4	-10352.9
0.4	-10618.4	-10755.7	-10830.7	-11022.6	-10614.2	-10700.8	-10409.5
0.5	-10585.5	-10734	-10775.7	-10968.4	-10569.4	-10641.2	-10358.9
0.6	-10597	-10728.9	-10789.1	-11007.6	-10555.5	-10634.8	-10387.4
0.7	-10562.8	-10729.8	-10779.5	-10989.1	-10567	-10635.2	-10386.1
0.8	-10585.5	-10712.9	-10771.6	-10976.4	-10549.3	-10633	-10358
0.9	-10583.7	-10728	-10810.1	-10977.5	-10554.2	-10651.3	-10347.7
1	-10583.2	-10711.4	-10766.9	-10978.8	-10541.8	-10656.9	-10381.7



a b

Fig. 1. Potential energy versus time step in MC simulation: a.Albumin-Water b.Albumin-Ethanol.

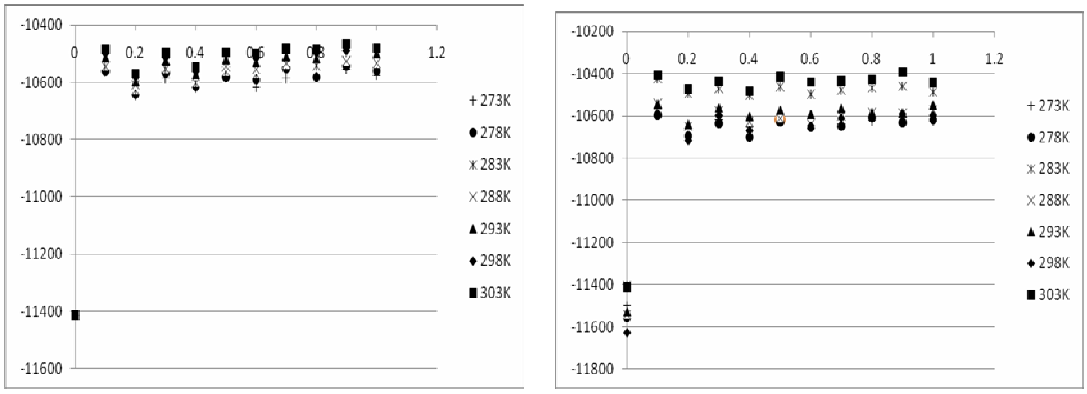


Fig. 2. Potential energy versus time step in MD simulation: a.Albumin-Water b.Albumin-Ethanol.

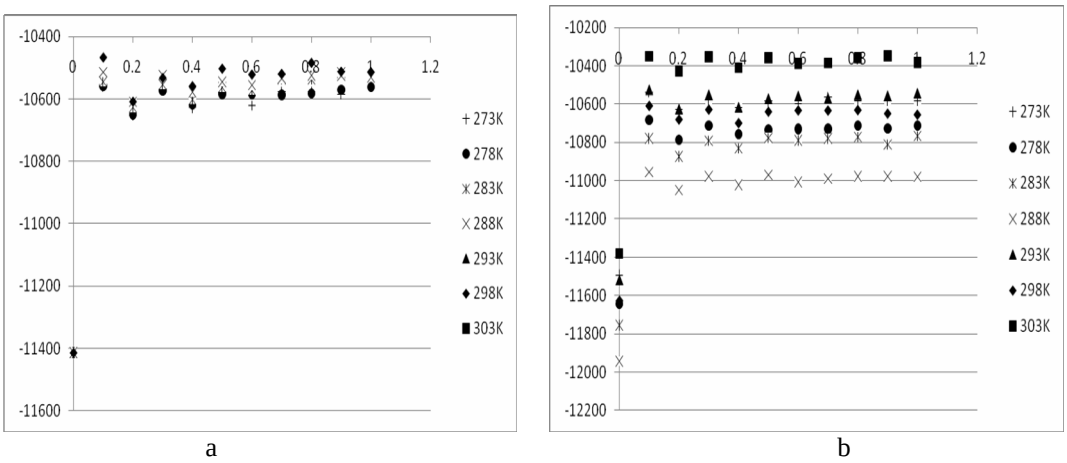


Fig. 3. Potential energy versus time step in LD simulation: a.Albumin-Water b.Albumin-Ethanol.

Fig. 1 displays the calculated results by MC force fields. It can be seen the changes of energy by time steps are not in the same way by increasing in initial temperature. It is understandable that there is a regular change in potential energy versus time step by increasing initial temperature in MD and LD simulation techniques in fig. 2 and fig. 3. This difference is because of the techniques used in simulations.

To apply the Monte Carlo method usefully it is necessary to (i) understand the statistical errors[18,19], and (ii) to ensure that enough Monte Carlo steps are done that the system has come to thermal equilibrium [20,21].

Molecular dynamics and Langevin dynamics simulations are important tools for understanding the physical basis of the structure and function of biological macromolecules. The early view of proteins are relatively rigid structures has been replaced by a dynamic model in which the internal motions and resulting conformational changes play an essential role in their function[22-30].

In macromolecules, MC simulation is not suitable for calculating energy because the statistical errors may occur more in macromoleculs and since Albumin is a macromolecule, so this irregular trend is obtained.

Tables 7,8 and figs4,5 report obtained potential energies versus temperatures. Also with analyzing fig. 4 and fig. 5, it is obvious that by increasing temperature, the potential energy decreases. Because in MD and LD methods, the system under studying is microcanonic and total energy in such systems is constant. So by increasing temperature, the kinetic energy increases and since total energy should be constant, consequently potential energy decreases.

Table 7. Obtained potential energies in different temperature calculated by MD method in different solutions/(kcalmol^{-1})

Time step	Albumin - Water		Albumin - Ethanol	
0	Temperature	Potential energy	temperature	Potential energy
0.1	272.999	-11413.2	273	-11497.6
0.2	144.105	-10564.7	142.951	-10566.6
0.3	156.389	-10645.8	152.798	-10637.4
0.4	147.515	-10586.7	150.044	-10617.1
0.5	152.498	-10619.4	155.386	-10655.1
0.6	147.438	-10585.5	147.422	-10597.7
0.7	152.244	-10616.8	151.791	-10628.6
0.8	147.69	-10586.5	153.218	-10638.5
0.9	146.906	-10581	151.099	-10623
1	143.057	-10555.4	148.903	-10607.1

Table 8. Obtained potential energies in different temperature calculated by LD method in different solutions/(kcalmol^{-1})

Time step	Albumin - Water		Albumin - Ethanol	
0	Temperature	Potential energy	temperature	Potential energy
0.1	273	-11413.2	273	-11493
0.2	143.63	-10562	139.345	-10536.7
0.3	157.597	-10654	151.922	-10626.8
0.4	145.14	-10571.7	145.022	-10577.2
0.5	153.72	-10628.1	150.788	-10618.4
0.6	146.4	-10579.7	146.224	-10585.5
0.7	152.623	-10620.5	147.857	-10597
0.8	145.855	-10575.8	143.102	-10562.8
0.9	146.048	-10576.9	146.293	-10585.5
1	147.22	-10584.5	146.06	-10583.7

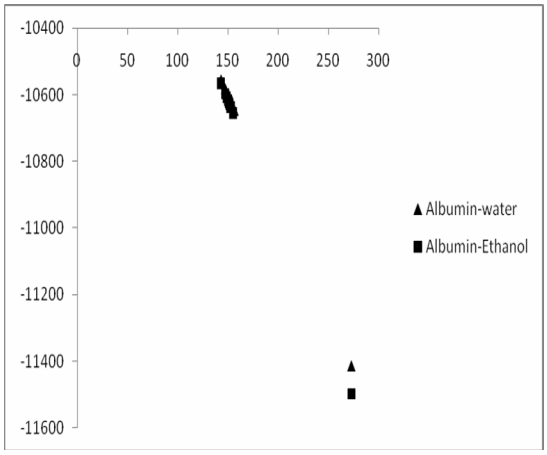


Fig. 4. Potential energy versus temperature in MD.

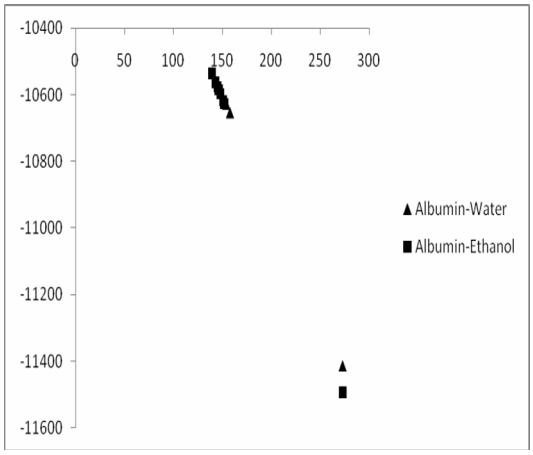


Fig. 5. Potential energy versus temperature in LD.

The optimized energy in Albumin - water system (-11413.2 kcal/mol) is more than optimized energy in Albumin-Ethanol system (-11497.6 kcal/mol) (table 7). So by changing solvent from Water to Alcohol, the system becomes more stable and its ability of transporting drugs and hormones becomes less. So Alcohol causes that Albumin to be poisoned.

Also by calculating $\Delta E_{kinetics}$ and its related ΔT, the values of heat capacity of Albumin in these solvents are calculated and are shown in table 9.

Table 9. Obtained energies versus temperature and Heat Capacity calculated by MD

reagent	Kinetic energy/ (kcalmol^{-1})	Temperature/ (K)	C/ (kcalmol^{-1} K^{-1})
Albumin/Water	1992.42	303.001	6.58
	1648.66	250.723	
	1326.84	201.781	
Albumin/Ethanol	2166.73	303	7.15
	1804.06	252.284	
	1429.49	199.903	

By adding of molecular mass of solvent, the value of heat capacity becomes more.

CONCLUSION

In this paper Albumin-Ethanol/Water solutions simulations are carried out by three methods including Monte Carlo (MC), Molecular Dynamic (MD) and Langevin Dynamic (LD) simulations in aqueous solutions. Investigation of the energy changes with time and temperature (between 273K to 303K) showed that MC technique is not suitable for macromolecule simulations. Also by comparing optimized energy in Albumin-water system and Albumin-Ethanol systems, it is distinguished that Albumin-Ethanol systems are more stable. So Ethanol can poison Albumin. Also the heat capacity of these systems is evaluated and it is shown that if the molecular mass of solvent increases, the value of heat capacity becomes more.

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