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The Effect of Monte Carlo, Molecular Dynamic and Langevin Dynamic Simulation and Computational Calculations on Insulin-like Growth Factor-1(IGF-1)

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Abstract

Insulin-like growth factor (IGF-1) is an anti-apoptosis factor in multiple cell types associated with various cancers. Computational methods allow investigating the systems between 50–100 atoms in the frame of quantum mechanics and up to 50,000 atoms with molecular dynamics. Since there are specific interactions between the residues, the solvent could play an important role in the stability of the native structure. Therefore it is useful to carry out such simulations at atomistic detail. MC, MD and LD simulations of the IGF-1 were performed with the HyperChem7.0 program. The geometries, and the interaction energies, bonds, angles, stretch-bends, electrostatic and the VDW Interactions were carried out in solution and gas phase. We have computed the transition temperature for the IGF-1 molecule. Studying the changes occurred in the potential energy of the three force fields showed that Amber force field is better than MM+ and OPLS force field and also MD simulation, at least in this model, is more effective than MC and LD methods. After equilibration, the MD simulation was very stable, and the difference between the relation coefficients R2=0.8173 in gas and R2=0.7558 in water was compared. The Pearson correlation suggests that there is an inverse relationship (R=-0.25) between *in vitro* temperature and stability of the structure.

***Keywords*:** IGF-1, AMBER, MM+, OPLS, MC, MD, LD

Introduction

Insulin-like growth factor (IGF-1), also known as somatomedin C, mediates the growth promoting activity of growth hormone. IGF-1 is autocrine regulator of cell proliferation, paracrine growth and survival factor for mammalian embryo development (Emmitte et al., 2009). Recent NMR studies have revealed that IGF-I has three α-helical regions surrounding a hydrophobic core (Laajok et al., 2000).

Over-expression or auto-activation of the insulin-like growth factor-1 receptor (IGF-1R) tyrosine kinase has been associated with various cancers. Insulin-like growth factor (IGF-1) is an anti-apoptosis factor of multiple cell types, and the anti-apoptotic effects are mediated through mitochondrial and cytochrome-c pathway (Li et al., 2003).

Development of faster computers that are within the reach of the widest scientific community as well as efficient computational methods allows investigating systems between 50–100 atoms in the frame of quantum mechanics and up to 50,000 atoms with molecular dynamics. Since the models become increasingly realistic, direct comparison with experimental data becomes possible (Na´ray-Szabo´and Berenteb, 2003) In addition to hit identification, docking techniques are increasingly used to support lead optimization efforts (Kitchen et al., 2004).

Recently, constant temperature molecular simulations of peptide folding have been reported using implicit solvent models, and explicit solvent models (Sung and Wu, 1997; Daura et al., 1998)

Recently, however, several computer simulations have demonstrated a strong coupling between hydrophobicity, solute-solvent dispersion attractions, and electrostatics. For example, simulations of explicit water between plate like solutes revealed that hydrophobic attraction and dewetting phenomena are strongly sensitive to the nature of solute-solvent dispersion interactions (Dzubiell, 2006). The competing effects of the

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solvent, such as the Van Der Waals (VDW) attraction and hydrogen bonding between the protein and solvent, reduce the strength of the interactions and consequently reduce the energy barrier related to the multiple minima problem (Ozkan, 2004).

In a solution, the intramolecular VDW interactions of a protein molecule are balanced by the intermolecular VDW interactions with solvent molecules. The possible difference between the protein intramolecular VDW attraction and that with water may be included in the hydrophobic interaction energy (Sung, 1999). Kurochkina and Lee have shown that the pair-wise sum of the buried surface area is linearly related to the true buried area (Sung, 1999).

Since the specific interactions between the residues and solvent play an important role in the stability of the native structure, it is useful to carry out such simulations at atomistic detail. This comes with the problem of timescale of folding/unfolding that is several orders of magnitude larger than those currently attainable by MD simulations (Ozkan, 2004).

Water plays a crucial role for the stability, dynamics, and function of proteins. For this reason, Molecular Dynamics (MD), Monte Carlo (MC) and Langevin Dynamic (LD) simulations must account for the effects that this solvent has, both on protein structure and on protein dynamics (Hamaneh and Buck, 2007).

The research group used structural bioinformatic methods to study the structure, function and dynamics of biomolecules. Our goal was to better understand structure formation of biomolecules and their mechanism. The function of proteins or nucleic acids depends on its three-dimensional structure and conformational dynamics. We employed the molecular dynamics simulation method as the main tool to study conformational dynamics of biomolecules. This approach allowed following dynamic molecular processes at high resolution in space and time. It was also possible to study the thermodynamics and energetics of structure formation and association. Our research was not restricted to a single biomolecular system.

The aim of the present work was to describe and characterize the molecular structure of IGF-1 crystalline-structure. In this work, the structure of a coordination compound modeling the IGF-1 was discussed computationally. Thus, it was worthwhile to collect information on its structure by means of computational chemistry as well.

Materials and Methods

Monte Carlo simulations are based on pair wise additive potentials of the form (Tafazzoli and Khanlarkhani, 2007). In concepts and algorithms of classical MD simulations the atoms of a biopolymer move according to the Newtonian equations of motion (Phillips et al., 2005)

For an all-atom MD simulation, one assumes that every atom experiences a force specified by a model force field accounting for the interaction of that atom with the rest of the system.

$$E_{total}=E_{bond}+E_{angle}+E_{dihedral}+E_{vdw}+E_{coulomb} \quad (1)$$

The Van Der Waals potential energy for the general treatment of non-bonded interactions is often modeled by a Lennard–Jones 12–6 function. We can consider an effective Hamiltonian operator constructed for molecule in a given geometry of it and the solvent:

$$H_{eff}=H_0+V_{elec}+V_{ind}+V_{non\text{-}elec}, \quad (2)$$

Where H_0 is the Hamiltonian in gas phase (the unperturbed Hamiltonian), V_{elec} is the perturbation from the permanent charge distribution of water, represented as a set of point-charges, V_{ind} is the perturbation from the induced dipoles in the solvent and $V_{non\text{-}elec}$ is a non-electrostatic perturbation, which models the effect of the anti-symmetry between the solute and solvent (Hermida-RamÓn et al., 2009)

Using Langevin dynamics, solvent effects can be modeled and the dynamic behavior of a molecular system in a liquid environment can be studied. These simulations can be much faster than molecular dynamics. These simulations can be used to study the same kinds of problems as molecular dynamics: time dependent properties of solvated systems at non-zero temperatures. Because of the implicit treatment of the solvent, this method is particularly well-suited for studying large molecules in solution.

Langevin dynamics simulates the effect of molecular collisions and the resulting dissipation of energy that occurs in real solvents, without explicitly including solvent molecules. This is accomplished by adding a random force and a frictional force to each atom at each time step (Berendsen, 1990; Karplus and Petsko 1990).

The random force is calculated as a random number, taken from a Gaussian distribution with a mean value of zero and with no correlation with the atom's velocity.

Molecular mechanics (MM) force fields rely on the combination of Coulomb and Lennard–Jones interactions to describe all non-bonded interactions (Ponder and Case, 2003). Even though the functional form of the potential energy is quite simple, it depends on a large number of empirical parameters, which must be obtained from *ab initio* calculations of the electronic structure in small molecules and/or experimental data.

Because each new term in the MM potential function requires additional empirical parameters, it is quite appealing to keep the functional form of the potential function as simple as possible. While most widely-used current force fields such as AMBER, OPLS do not employ explicit hydrogen bonding terms, this was not always the case (MacKerrell et al., 1998; Hagler and Lifson, 1974; Cornell et al., 1995; Jorgensen et al., 1996; Weiner et al., 1984).

The crystal structures of proteins were from the Brookhaven Protein Data Bank. The structure of protein IGF-1 was selected from the Protein Data Bank (PDB code 1B9G). These studies provided insights into the steric, electrostatic, hydrophobic, and hydrogen bonding properties and other structural features influencing the IGF-1.

In vacuum, the system was simulated using Monte Carlo, Molecular dynamic and Langevin dynamics with 100 ps step and without any constraints. Temperature was kept constant at 300 K. In water, simulations, the system was placed in a box (3 x 3 x 3 nm) containing one molecule of solute and 884 TIP3P water molecules (figure 1).The system was simulated using Newtonian dynamics with 100 ps step and no constraints applied to the solute.

The complex was solvated by added water molecules. The systems were first energy minimized steps with the conjugate gradient algorithm. Then, the position-restrained MC, MD and LD simulation were run 100ps. Afterwards, 1 ps simulations were carried out at a time step of 100ps (figure 1).

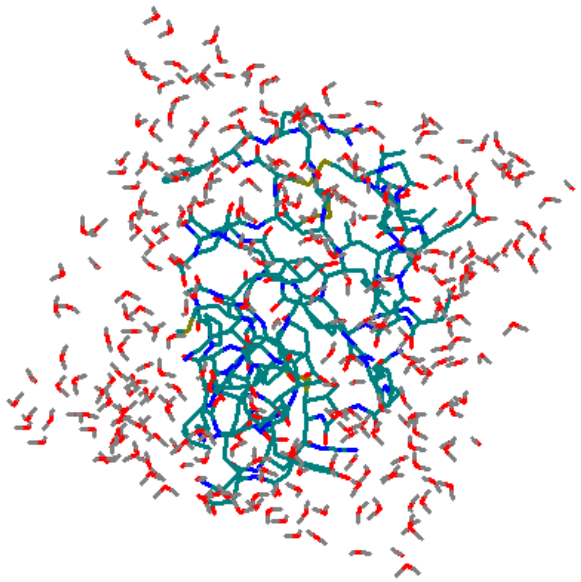

Figure 1. Schematic representation of structural model of IGF-1R in water (884 TIP3P water molecules).

Several simulations were carried out, as listed in Table 1. MC, MD and LD simulations of the IGF-1 were performed with the HyperChem7.0 program (HyperChem 7.0, 2001). The geometries, and the interaction energies, bonds, angles, stretch-bends, electrostatic and the VDW interactions were carried out in solution and gas phase (table 1, figure 2).In the simulation of the small water system, with temperature of 295, 297, 299, 301, 303 and 305 K, the instantaneous kinetic temperature is given by Eq (3), (Español and Warren, 1995):

$$Tk\,(t) = \sum_{i=1}^{N} m_i v_i^2(t)/k_B N_f \qquad (3)$$

Where k_B is the Boltzmann constant, N_f is the degrees of freedom ($N_f = 3N - 3$ for a system of N particles with fixed total momentum), m_i is the atom weight for atom i, v_i is the velocity of atom i. The effect of confinement on the thermodynamic properties of several statement proteins was investigated by performing simulations over a large range of temperatures. We have computed the transition temperature for the IGF-1 molecule.

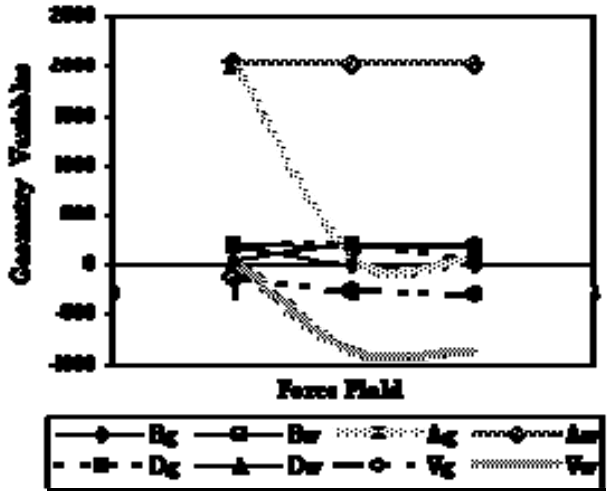

Figure 2. Geometry of optimized variables of Bond length (B), Bond Angle (A) and Dihedral Angle (D) in gas and water media at 300K.

Table 1. Calculation of various variables in 300 K Temperature for IGF-1 at MM+, AMBER, OPLS.

	GEOMETRY						MC	MD			LD		
environment	Force Field	Bond	Angle	Dihedral	Energy	Gradient	Potential	Potential	Kinetic	Total Energy	Potential	Kinetic	Total Energy
gas	MM+	174.408	2003.05	187.056	2186.66284	0.099337	547.949	2456.24	386.747	2842.99	354.473	388.198	742.671
	AMBER	11.0393	67.3022	200.754	23.23151	0.099951	2798.14	348.117	393.388	741.505	2481.28	394.672	2875.95
	OPLS	2.53763	47.7753	48.4863	-202.51639	0.098917	349.788	138.21	390.617	528.827	131.714	391.538	523.253
water	MM+	202.777	2037.84	43.0804	-130.11968	0.095515	2217.07	6677.62	9695.98	16373.6	672.07	1562	2300.2
	AMBER	175.662	2019.97	212.994	270.345123	0.084089	160.52	733.188	1608.57	2341.76	746.05	1596	2342.05
	OPLS	175.662	2019.97	212.994	270.345123	0.084089	1367.75	686.563	1826.73	2513.3	641.945	1871.01	2512.95

Results

The following text describes methods for generating and evaluating representative molecular conformations, particularly for peptides and small proteins, based on Molecular Mechanic energy functions. On the other hand, Molecular Mechanics describe molecules as atoms linked with springs (harmonic bond stretches and bond angle wagging), each atom having finite volume and relatively sharp boundaries ("6-12" hard spheres potentials), with sinusoidal torsion energies. The force field for a typical protein can be given as a sum of the various components including bond stretching and bending, torsion potentials, and non-bonded interactions.

Molecular dynamic simulations have been widely used to obtain the 'real' bioactive conformation when the crystal structure of protein–ligand complex is unavailable. So, in order to obtain the 'real' stabilized bioactive compound was used for molecular dynamic simulations. The system was well-equilibrated and 500 ps in the range of the MD equilibration were selected for further processing analysis. We have shown relation coefficients in figure 3.

In this paper, we have used Monte Carlo methods to study IGF-1 in the bulk and in a confined environment. Results are presented in table 1 in respect of the effects on the specific media of the structure.

Simulations of molecular dynamics were carried out on the two systems, gas and solvent IGF-1 molecules. All simulations were carried out at the constant temperature. All simulations were performed at 300 K. Each solvent system was immersed in a periodic water box, and the structures of water molecules were maintained. A 100 ps time step was used in all simulations.

Furthermore, we used MD and LD methods to study protein in the bulk and in confined media. The structures obtained throughout this calculation were optimized using MM+, AMBER, and OPLS force field parameters. Also, all these approaches included discrete particles moving in a defined energy landscape according to Langevin Dynamics (LD).

Table2. Calculation of Energy potential (kCal/mol) in various Temperatures for IGF-1 at MM+, AMBER, OPLS.

	EPOT(MC)						EPOT(MD)						EPOT(LD)					
T(K)	MM+		AMBER		OPLS		MM+		AMBER		OPLS		MM+		AMBER		OPLS	
295 297	4235.984	2888.215	537.0323	563.3023	354.1178	328.5373	4210.79	4288.985	527.3578	535.2763	354.2476	318.2481	2579.846	3405.258	513.2186	528.15	321.3818	311.2994
299 301	2906.046	2900.473	557.4321	535.0936	345.7581	343.7589	3419.356	3440.532	494.5307	495.9955	319.1986	341.9576	2569.473	3506.185	518.6368	508.0626	343.8734	339.2246
303 305	2908.884	2917.52	554.7775	526.3257	347.554	331.7875	3455.45	3483.999	498.6193	497.0406	233.3117	377.6465	3480.613	3450.559	539.5172	502.6813	316.3417	311.1847
295 297	4235.91	2883.358	545.3885	542.0403	350.5241	320.7311	4290.329	4320.816	502.2061	519.6428	339.9279	310.6455	2589.535	3444.03	499.7869	516.0245	322.7999	308.6239
299 301	2887.079	2885.448	553.002	541.189	354.173	349.8519	3434.814	3439.83	526.4235	493.8266	336.4753	291.9342	2578.557	3467.769	543.1108	501.8044	326.8775	332.2738
303 305	2900.525	2923.604	554.0753	527.549	351.5697	340.6064	3443.293	3416.052	480.1488	504.6467	236.4355	320.6327	3495.918	3421.9	530.5234	482.4206	305.1587	298.222
295 297	4235.836	2871.213	550.2813	555.388	345.1127	331.0986	4371.666	4350.692	525.0994	507.8335	341.3153	288.8688	2567.801	3432.501	508.1079	497.8874	322.6342	343.9134
299 301	2873.877	2883.255	549.6397	547.9619	352.4398	343.5526	3386.889	3478.571	529.2417	533.0989	312.8945	301.8429	2546.555	3458.191	502.9492	500.4421	339.418	332.5635
303 305	2889.936	2912.401	558.5192	519.8594	344.3478	345.3649	3460.487	3472.971	490.9563	517.9937	231.1382	344.6291	3428.675	3470.391	479.7136	493.0887	346.9156	310.3809
295 297	4235.662	2858.414	543.9048	543.5708	345.1661	326.7686	4296.785	4329.588	504.7312	505.6049	336.4077	341.1394	2588.488	3409.925	512.786	496.3951	328.6388	351.7402
299 301	2869.987	2880.593	554.9779	555.7316	362.0574	350.8972	3479.096	3495.251	522.251	542.6166	299.5432	336.3144	2568.802	3477.177	544.3472	502.4798	363.9548	315.3748
303 305	2888.042	2902.244	567.5231	523.7003	342.4099	347.168	3462.17	3476.277	511.3409	509.614	213.2367	333.0184	3455.711	3486.662	507.2958	500.5871	296.7043	329.4997
295 297	4235.785	2864.459	549.2016	542.6379	346.0615	333.429	4303.447	4371.86	487.4169	537.4854	302.3023	317.8343	2590.098	3408.431	505.4064	508.2617	301.2283	351.383
299 301	2879.702	2870.632	571.1646	551.1309	377.0245	361.2684	3489.193	3518.701	496.4862	513.6497	313.907	281.5862	2552.44	3465.14	505.245	521.7915	320.9432	343.0555
303 305	2868.186	2899.195	566.4869	513.8336	347.6069	330.4477	3371.318	3426.49	528.2641	502.7837	198.5066	310.0454	3507.042	3430.872	525.1266	502.9998	311.7542	315.791
295 297	4235.928	2860.041	545.8426	541.5521	355.5735	331.6525	4300.576	4355.381	500.8381	516.929	319.1307	320.479	2577.903	3472.735	482.6723	524.14	304.7759	328.6955
299 301	2867.552	2873.999	560.8979	549.9072	383.4842	363.6535	3505.641	3453.456	499.9372	516.7252	334.0543	294.9457	2577.913	3479.335	503.0349	489.6058	323.2028	322.9286
303 305	2860.671	2886.921	569.0027	526.6578	348.5785	326.4272	3409.008	3444.407	506.0706	524.8028	214.6883	347.8624	3503.99	3469.163	492.9588	479.1641	317.0148	326.6403
295 297	4235.977	2838.857	547.4008	542.6965	354.9743	334.1456	4309.237	4265.458	513.5359	550.3824	345.2705	332.0029	2585.024	3409.999	501.673	551.8837	305.6494	353.8895
299 301	2856.91	2850.952	554.6624	559.3318	382.8443	358.8365	3514.08	3402.411	525.3257	539.2837	322.789	347.5192	2573.96	3472.471	548.2233	507.2563	333.6927	333.4404
303 305	2863.217	2878.897	555.89	523.1423	347.6105	341.3449	3466.064	3413.669	535.2093	489.278	203.4976	319.6483	3472.611	3439.305	501.8165	489.4213	313.1117	316.1416
295 297	4236.079	2847.769	563.6295	555.1603	358.1401	326.2686	4401.925	4443.53	463.4955	524.0253	330.9887	307.1991	2563.826	3454.12	524.3984	503.3033	341.5131	350.8395
299 301	2841.119	2853.838	549.9271	540.7308	378.2664	366.259	3421.173	3422.556	508.1931	522.0677	340.3782	304.4567	2565.485	3459.745	506.735	507.7916	305.9	345.6635
303 305	2865.166	2867.208	561.7354	541.0972	340.2723	334.8615	3370.795	3468.461	468.5343	473.9662	208.2185	330.4919	3474.678	3488.03	514.5964	520.7366	294.8215	293.1924
295 297	4235.939	2841.011	559.6968	550.5739	350.0228	341.2642	4284.895	4278.622	527.4733	523.5819	208.2185	330.4919	2569.188	3385.981	490.5222	519.7032	324.46	297.4873
299 301	2854.111	2854.219	551.3961	543.2766	376.6521	359.0934	3415.884	3449.611	511.243	528.6366	331.4785	338.7397	2564.208	3433.359	525.5302	504.838	305.8051	320.024
303 305	2851.653	2869.821	553.7677	543.8042	346.1711	347.44	3484.139	3482.982	493.9665	497.0681	178.9832	351.4939	3541.201	3426.485	495.4621	527.0049	349.1488	325.338
295 297	4235.915	2840.159	558.8617	543.8674	348.7244	342.525	4345.033	4310.343	465.7351	514.3511	301.7872	348.601	2559.526	3414.71	489.266	505.9484	314.4352	313.0575
299 301	2864.091	2847.136	548.6024	533.6068	366.5257	355.267	3421.798	3485.754	529.0999	481.7465	324.3557	322.1682	2566.563	3401.513	529.5195	500.1281	321.0137	356.852
303 305	2857.226	2870.121	549.1909	544.1115	348.4853	346.989	3461.478	3515.989	517.4446	522.877	204.1998	349.8143	3476.94	3474.156	467.2895	473.4712	297.3159	301.3034
295 297	4235.679	2850.142	563.642	544.8266	357.2484	339.673	4215.188	4384.653	499.0536	501.712	317.7411	325.0367	2584.529	3460.82	516.8234	517.6833	319.8571	346.1244
299 301	2859.588	2843.769	551.4084	531.3312	359.5042	345.7718	3530.324	3437.044	533.8555	533.4912	349.8931	324.2932	2569.737	3443.905	490.1534	502.6556	337.8195	358.6917
303 305	2854.746	2866.983	545.3275	546.4106	340.9888	354.5321	3507.608	3431.513	516.1681	508.7436	219.8929	325.5009	3448.999	3474.151	512.2114	473.2974	285.3812	328.9042
295 297	4235.633	2844.473	564.7132	533.1169	363.6589	339.187	4330.001	4281.668	500.4392	531.2556	307.7915	311.8912	2574.05	3398.087	492.1332	511.4382	345.2494	311.3421
299 301	2853.499	2840.504	546.4174	535.2596	369.4149	327.8606	3462.701	3451.626	507.1952	527.718	333.3354	334.4424	2561.146	3397.049	511.8376	504.886	328.7564	346.241
303 305	2848.781	2860.427	554.335	547.3047	343.462	350.2364	3454.264	3422.529	485.2639	484.4238	213.4292	321.2014	3448.117	3429.749	489.7065	538.1508	346.3618	314.3207
295 297	4235.695	2835.124	550.9998	512.8999	360.4237	345.0831	4303.186	4365.847	483.8532	518.2829	318.0182	306.6712	2571.361	3429.874	510.5202	482.568	342.7836	316.2462
299 301	2842.188	2833.22	536.8683	539.688	363.5614	329.5917	3394.981	3489.914	496.1218	522.541	310.7109	345.0437	2531.758	3435.588	531.8323	474.8859	338.4859	297.9369
303 305	2841.851	2857.681	542.8929	548.7379	359.5547	354.4125	3377.382	3447.376	469.0301	522.9171	197.4162	299.3484	3463.164	3457.529	500.7173	546.606	338.8057	290.12
295 297	4235.842	2834.423	542.9478	516.5182	352.2798	337.6797	4326.629	4445.121	520.0923	518.8304	319.1497	344.6331	2569.537	3467.652	527.666	484.2487	285.2081	339.9539
299 301	2847.525	2822.857	534.5339	537.8507	360.5002	332.4513	3514.473	3476.453	506.4193	543.2968	318.6115	339.1389	2562.692	3396.74	500.7725	499.8272	292.3938	340.2541
303 305	2839.497	2856.229	541.7733	554.9586	368.2707	349.5836	3447.624	3484.363	513.571	491.1841	169.4245	326.2012	3448.279	3472.96	494.8175	498.7156	337.9184	359.5291
295 297	4235.807	2842.41	548.394	517.0762	348.838	342.7613	4366.921	4358.478	534.6727	537.1	331.8006	324.4326	2569.649	3450.92	482.1576	514.1124	253.1677	332.026
299 301	2852.654	2818.777	541.3942	551.8688	350.7536	339.3084	3467.887	3434.047	533.4216	519.5013	318.7386	337.4207	2573.344	3493.333	499.376	516.9822	342.8565	304.5523
303 305	2845.101	2859.85	549.7361	545.2499	363.6275	340.7894	3418.719	3461.921	495.7306	530.857	201.4282	296.0907	3371.327	3464.815	504.6794	524.4158	332.0623	336.4991
295 297	4235.856	2828.16	561.4	517.4912	339.2717	339.9656	4358.029	4280.032	515.1163	520.4341	301.7409	311.0852	2570.01	3453.387	472.2345	495.7901	294.4622	326.0108
299 301	2846.003	2823.927	547.8884	559.1285	359.7528	326.1819	3453.021	3500.615	544.1592	520.9449	313.7343	343.6175	2552.84	3438.135	513.3206	506.941	328.7103	324.6017
303 305	2846.762	2846.006	541.671	557.965	359.8163	337.309	3476.481	3478.572	485.7271	520.3431	205.9013	319.3591	3472.448	3493.887	512.1343	510.7655	354.5991	314.9204
295 297	4235.738	2813.885	558.9424	524.2564	339.6264	340.827	4341.226	4272.062	519.3115	529.032	342.0903	342.3374	2555.729	3441.786	516.4899	492.0165	325.7104	320.921
299 301	2846.063	2826.407	544.5283	541.882	354.9368	329.647	3469.677	3453.05	503.5554	532.517	320.2513	302.2707	2564.157	3449.956	523.1369	507.9351	312.8271	328.0562
303 305	2835.018	2859.977	555.5255	565.3812	352.9486	358.4168	3474.609	3393.49	498.4059	495.9245	213.9004	321.8973	3387.85	3468.916	503.0721	538.5077	337.9524	350.9694
295 297	4235.683	2820.807	574.8336	523.1565	333.9953	335.1254	4302.709	4355.351	503.8653	543.6403	337.7553	324.653	2571.258	3404.964	505.9321	515.333	335.3871	309.2835
299 301	2848.388	2827.333	540.8102	542.011	365.9344	333.2373	3460.494	3440.337	516.6548	499.4484	338.2431	334.1742	2571.988	3465.41	498.7625	485.5512	299.1711	319.1378
303 305	2837.379	2853.203	545.1721	574.3134	354.3969	360.9447	3425.421	3445.563	533.1451	497.0183	195.717	353.9156	3392.038	3458.916	499.8112	482.5093	345.6826	332.3725
295 297	4235.73	2828.653	563.7302	517.9006	335.5606	330.472	4452.299	4335.674	490.5256	501.5159	299.9477	311.4561	2562.191	3446.998	522.1642	542.3101	265.5349	299.0892
299 301	2851.181	2836.091	529.7856	549.5345	360.0608	338.0183	3417.058	3488.196	528.3224	502.3401	342.9102	355.2545	2563.942	3523.837	459.5745	528.3569	353.7659	329.5145
303 305	2842.026	2848.864	548.3592	572.5765	350.7377	370.9806	3449.055	3537.28	501.5012	495.1578	205.6	353.6971	3438.471	3486.51	519.3807	493.3634	310.4973	320.9409
295 297	4235.575	2819.025	569.4018	522.2225	329.4926	326.7581	3449.055	3537.28	509.4262	490.1977	338.5802	328.9518	2573.969	3433.049	488.3241	487.7672	276.904	335.0679
299 301	2828.133	2830.444	516.1687	546.084	347.6144	330.1531	3496.024	3448.752	538.645	507.7447	315.2097	322.3077	2570.754	3442.332	489.7299	491.5191	312.0651	310.3455
303 305	2820.963	2838.694	552.3593	568.3253	360.2547	367.6311	3449.451	3443.293	497.5652	540.6433	201.1053	319.8668	3459.938	3453.697	455.7375	528.3239	309.7843	332.9762
295 297	4235.474	2810.148	569.616	532.5905	341.4251	323.0252	4328.744	4388.186	484.329	487.0341	338.1252	320.3201	2573.179	3486.304	503.837	492.3624	316.411	330.6078
299 301	2824.638	2821.123	520.2739	557.0218	345.387	320.7092	3508.281	3437.637	507.1878	516.707	318.6565	307.1662	2562.767	3451.816	518.1656	496.1881	342.388	322.0578
303 305	2809.159	2837.105	550.9778	559.1342	350.4919	363.2806	3455.588	3481.294	482.9492	503.6921	206.5169	330.3023	3392.909	3466.878	497.1192	480.7999	310.8878	374.3974

We used over many instantaneous values to get an accurate estimate of the temperature. These methods, which rely upon uniform sampling of energy space, can yield thermodynamic data over the entire temperature range of interest and showed to overcome large free energy barriers. We have reported findings for six different temperatures of various sizes and topologies. Results are presented in table 2 in which potential energies of IGF-1 are indicated in various temperatures. The results are summarized in table 1 for 300 K in gas or solvent and in table 2 for 295, 297, 299, 301, 303 and 305 K temperatures. The theoretically possible stable conformers of free molecules were examined by means of a molecular dynamic calculation performed in a temperature interval from 0 to 500

K. For example, by the iterative calculation with time step of ‘‘100 ps”, using ‘‘Chem3D” software, the experimental X-ray geometrical data reported for IGF-1 in crystalline structure, were used as input geometrical data (Hartung et al., 1992). At the next step, the appropriate ones, carefully selected from the structures obtained throughout this calculation, were optimized using MM+, AMBER and OPLS force field parameters and included into the same software. In this paper, a comprehensive conformational research on free molecules was carried out.

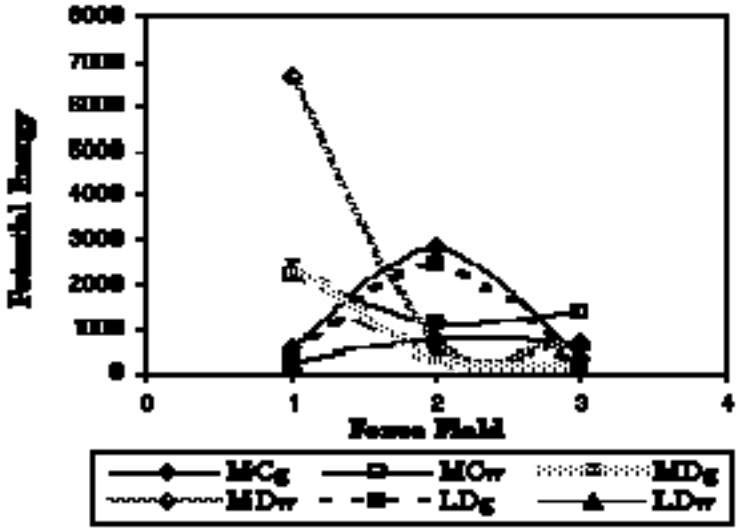

Figure3. The potential energy (kcal/mol) via time (ps) during Molecular Dynamic (MD) simulation at 300K in gas (R2=0.8173) and water (R2=0.7558) environments to a stabilized structure of IGF-1.

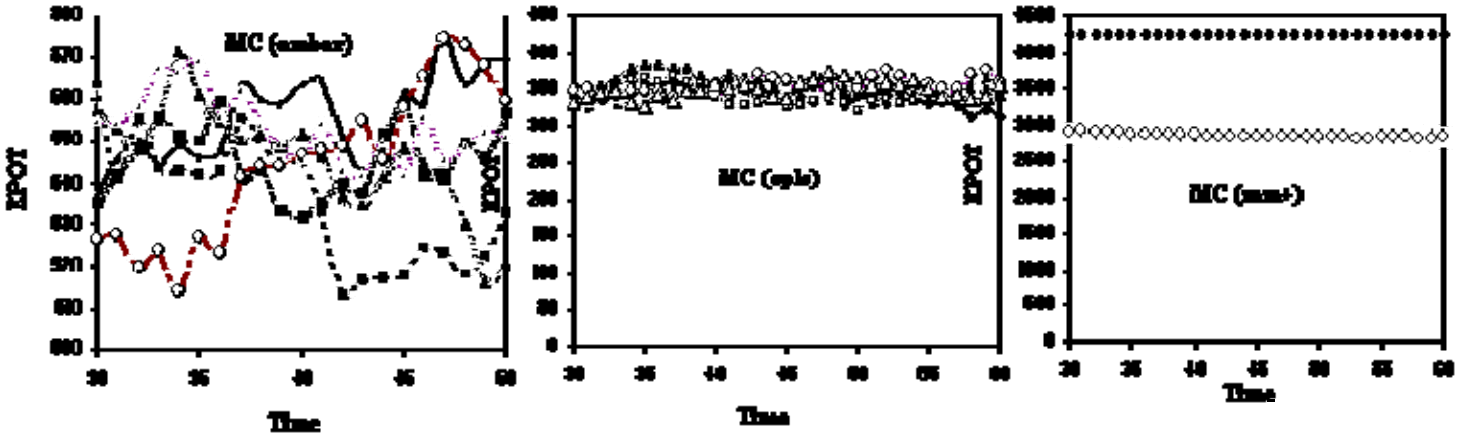

Figure 4. The potential energy(kcal/mol) via time (ps) during Monte Carlo (MC) simulation at 295,297,299,301,303 and 305 K using **(a)** AMBER (**b)** OPLS **(c)** MM+ force fields corresponding to a stabilized structure of IGF-1.

Potential energies for the three force fields of MM+, AMBER and OPLS at Monte Carlo simulation were compared in figure 4. The average energies are in good agreement with the simulation accuracy. The sampling results of step-size of MD and LD methods are presented in figures 5 and 6, respectively.

Discussion

In conclusion, in this work we used molecular dynamic models to explore the stability of IGF-1 by comparing theoretical methods of simulation. A highly selective effect of temperature and environment was discovered in chemical structure and it investigated the standard constant

temperature at MC, MD and LD simulations. Molecular Dynamic simulation method has been employed as the main tool for studying conformational dynamics of biomolecules.

One of the force fields designed for treating macromolecules can be simplified by not considering explicitly – the so-called united atom approach is AMBER. Seemingly, solvent effects influence the calculated potential energy surface by lowering potential energy barriers on angle. This means that the parameterizations that have been developed for small molecules with considerable effort can be carried over into macromolecular calculations with little or no change. Also, the MM+ and OPLS force fields parameters were applied for IGF-1 model in gas and for water environment.

Also, the possible difference between the IGF-1 intramolecular VDW attraction and that with water were included in the hydrophobic interaction energy. The short- range repulsion represents the exclusive volume of each atom and needs to be calculated explicitly.

In a solution, the intramolecular VDW interactions of a protein molecule are balanced by the intermolecular VDW interactions with solvent molecules. Thus, when solvent molecules are not explicitly included, the intramolecular VDW interactions must be adjusted accordingly. The longer-range attractive VDW interactions provide a nearly uniform background potential (Chandleret al., 1983), and therefore can serve as the reference for the VDW energy calculation (McCammon et al. 1980).

The measurement of the potential of solvation under similar conditions of temperature in solution along with investigation of energetic and structural aspects of solution were used to gain insight into the molecular level interaction with IGF-1. Solute–solvent pair interaction of potential energies show that the greater stability of solvent observed over all states investigated in this study is related to the MD/AMBER approach. The obtained results have demonstrated that the free molecule has a very flexible macro-cyclic structure. On the basis of the theoretical results obtained for the determined most stable, the dependencies of the geometrical and force constants parameters of the free molecule to its conformational structure were discussed.

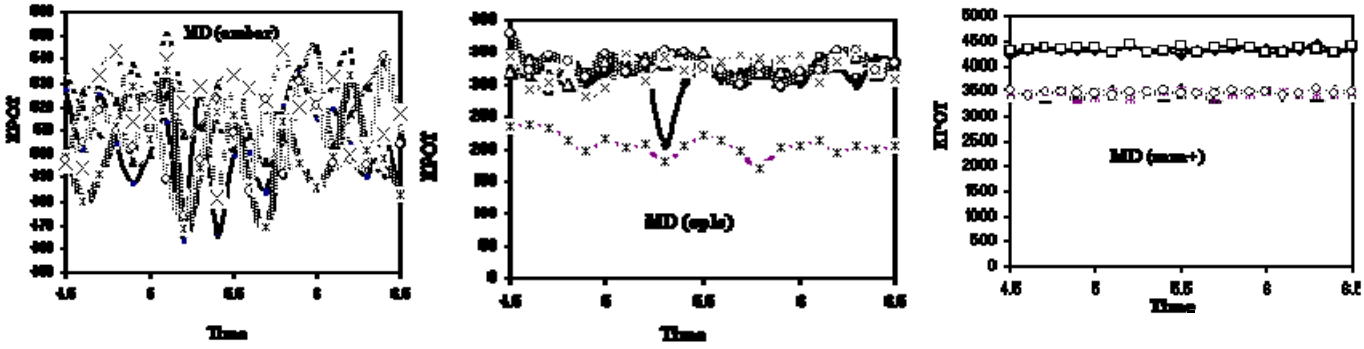

Figure 5. The potential energy (kcal/mol) via time (ps) during Molecular Dynamic (MD) simulation at 295,297,299,301,303 and 305 K using a) AMBER b) OPLS and c) MM+ force fields corresponding to a stabilized structure of IGF-1.

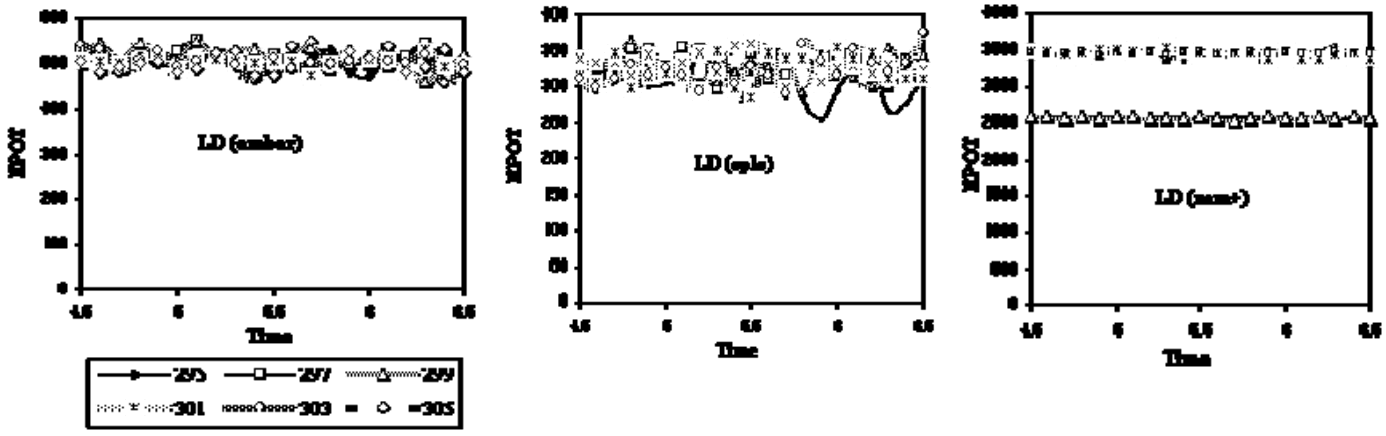

Figure 6. The potential energy (kcal/mol) via time (ps) during Langevin Dynamic (LD) simulation at 295,297,299,301,303 and 305 K using a) AMBER b) OPLS and c) MM+ force fields corresponding to a stabilized structure of IGF-1.

The potential energy, represented through the MD "force field" is the most crucial part of the simulation since it must faithfully represent the interaction between atoms, which is to be cast in the form of a simple mathematical function that can be calculated quickly. After equilibration, the MD simulation was very stable; therefore we tried to compare the difference between the relation coefficients R2=0.8173 in gas and R2=0.7558 in water. These results show that the force field of AMBER has a convenient relation in all simulation methods as well as in various media (figure 3).

As expected, AMBER demonstrates much smoother energy profiles than the other two simulation methods due to higher-order energy conservation in the modified Hamiltonian (figure 4a). The magnitudes of energy fluctuations in both MM+ and OPLS approaches are significantly smaller than the other (figure 4b, c). Observed data are almost identical for both choices of the MD simulation length suggesting that the MD simulation had been affected much more on acceptance rate - at least for this particular model - than MC and LD approaches. This potential does not have any terms describing angular dependencies of hydrogen bonds and is similar to the 10–12 hydrogen bonding potential originally proposed by others (McGuire et al., 1972). They found that hydrogen bonding energies were represented adequately by a sum of Lennard–Jones and electrostatic interactions plus the 10–12 hydrogen bonding term with empirical constants adjusted according to the hydrogen bond type.

Because the functional form of such a hydrogen bonding term was very close to the Lennard–Jones component of the force field, the second-generation AMBER force field omitted it altogether (Cornell et al., 1995), relying instead on the combination of Lennard–Jones and Coulomb interactions to model hydrogen bonded complexes, thus the data of this force field in three simulation methods showed that the changes of potential energy via time at various temperatures are much better than MM+ and OPLS force fields (figure 4a, 5a, 6a).

Similarly, the widely used OPLS force field does not contain an explicit hydrogen bonding term: the emphasis of OPLS parameterization is on reproducing thermodynamic properties of organic liquids such as enthalpies of vaporization, densities and free energies of hydration (figure 4b, 5b, 6b) (Jorgensen, 1996; Jorgensen and Tirado-Rives, 1988).

Because each new term in the MM+ potential function requires additional empirical parameters, it is quite appealing to keep the functional form of the potential function as simple as possible (figure 4c, 5c, 6c).

MD simulation is suitable for obtaining the elastic properties of a system in our size. For certain confining environments, individual proteins do exhibit power-law dependence, but the relationship is different for each molecule. In other cases, the increase in stability upon confinement interestingly demonstrates no monotonic behavior. ANOVA showed that there is a significant difference between the differences in the potential energy in the amber force field and MM+ and OPLS (P-value=0.035). Furthermore, the Pearson correlation suggests that there is an inverse relationship (R=-0.25) between in-vitro temperature and stability of the structure.

These results also revealed that the solvation of IGF-1 is the major component for the interaction potential energy and it was clearly shown that the role of the solute-solvent interactions is more pronounced in IGF-1 solvation. The major part of this difference is due to the interaction of IGF-1 with solvent molecules corresponded to various simulation methods and force fields. A difficult task in the computational study of stabilized structure is to find a proper energy function that can lead to a unique structure. Our simulations showed that the simple energy function modified to include solvent effect has a parameter range that can simulate indicated structure at constant temperature of 300K.

Several molecular dynamic simulations could be performed over a wide range of temperatures, and the data could be combined using a weighted histogram approach (Weiner et al., 1984); however, the statistical error associated with the tails of the sampled distributions is usually large and can propagate when data from simulations at different temperatures are merged.

From the simulations, it is demonstrated that the kinetic temperature of the system is properly bounded around the prescribed equilibrium temperature. The length of each simulation was 100 ps. The relative drift of molecular temperature denoted by ΔT in percent, with respect to mean temperature; T in Kelvin has been measured. Figures showed the function of the reduced temperature. Low reduced temperatures promote complex structure stability, whereas high reduced temperatures oppose it.

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