Journal of Physical and Theoretical Chemistry
of Islamic Azad University of Iran, 5 (3) 161-166: Fall 2008
(J.Phys.Theor.Chem.IAU Iran: Fall 2008)
ISSN: 1735-2126

Molecular Modeling Studies on Vinblastine Binding Site of Tubulin for Antimitotic agents

Z.Varmaghani[1], F. Mollaamin[2*], L.Pishkar[3], B.Khalili Haddad[4]

1. Department of Biology, Science and Research Campus, Islamic Azad University, Tehran, Iran
2. Department of Chemistry, Qom Branch, Islamic Azad University, Qom, Iran
3. Department of Biology, Islamshahr Branch , Islamic Azad University, Islamshahr, Iran
4. Department of Biology,Roodehen Branch, Islamic Azad University, Roodehen, Iran

ABSTRACT

Medicinal chemistry depends on many other disciplines ranging from organic chemistry and pharmacology to computational chemistry. Typically medicinal chemists use the most straightforward ways to prepare compounds. The validation of any design project comes from the biological testing.

Studies of the binding site of vinblastine by a single cross–linking experiment identified it as being between residues 175–213 in β–tubulin.These polypeptide residues are in the region of lateral or longitudinal contacts of protofilaments on the microtubule in the absence of a ligand. In the presence of vinblastine or other active vinca alkaloids a kink is formed that disturbs normal microtubule formation and favors depolymerization.

In an effort to understand the conformational preferences that may be attributed to stereoelectronic effects, a number of computational chemistry studies carried out. Molecular mechanics, Monte Carlo, Molecular Dynamics and Langevin calculations using the AMBER force field performed on vinblastine .These results show the minimized structure of vinblastine, calculated potential energy for important dihedral angles, and the effect of temperature on geometry of optimized structure.

Keywords: microtubules; vinblastine; Monte Carlo; Molecular Dynamics; Langevin; simulation

INTRODUCTION

Many drugs currently used in cancer chemotherapy cause mitotic arrest by interacting with tubulin and suppressing microtubule dynamics.Microtubules are a major cytoskeletal element,essential in segregation of chromosomes during cell division, axonal transport,secretory processes, and the maintenance of specific cell morphology. Conditions that promote or diminish tubulin assembly into microtubules prevent separation of chromatids and subsequent formation of daughter cells. The vinca alkaloids inhibit assembly of microtubules at substoichiometric levels [1].

Vinca alkaloids are antimitotic, anticancer agents that induce tubulin to form spiral polymers at physiological protein concentrations.

We usedsedimentation velocity to investigate the effects of six vinca alkaloids on tubulin

* . Corresponding author:smollaamin@gmail.com

spiraling. Spiraling potential is an indicator of antimitotic activity in vivo, although turbidity studies indicate that there is no correlation between spiraling potential and microtubule inhibition in vitro. Mechanisms that explain this discrepancy are discussed[2].

Vinblastine(VLB)[3] and its congeners,dimeric indole alkaloids derived from catharanthus (Vinca) roseus,are highly useful drugs for the treatment of certain malignancies.

Vinblastine can bind to dimeric tubulin, to microtubule ends, and to certain aggregates formed by self-association of tubulin. This has led to considerable confusion in the literature regarding the affinity constants, which are stated to vary over several orders of magnitude. These values are influenced by the solvent composition, protein concentration, presence or absence of associated proteins, and method of assay [4 ,5].

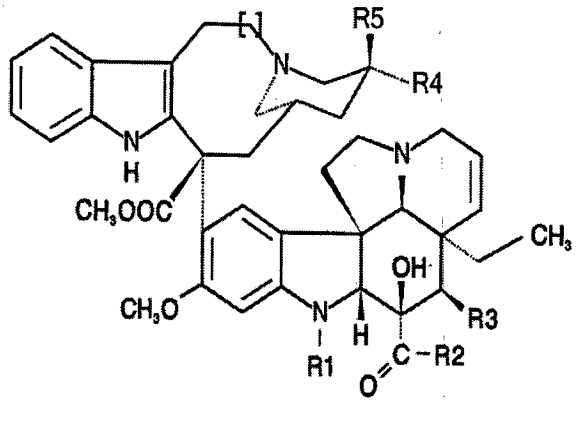
The interactions of vinblastine with tubulin heterodimers and microtubules have been studied extensively,and in vitro studies have shown that at low ionic strengths vinblastine induces spiral formation by a mechanism involving ligand-mediated plus ligand-facilitate isodesmic self association

Molecular mechanic simulation method are specially useful in studying systems with a large number of coupled degrees of freedom, such as liquids disordered materials, strongly coupled solids and cellular structures. These methods are very important in physical chemistry particularly for simulations involving atomic clusters [6] . The aim of this work is to understand molecular mechanic of vinblastine drug, which will be useful for designing anticancer drugs. Here in we used molecular mechanic simulation, within the Monte carlo/ Langevin dynamic/ Molecular dynamic (MC/LD/MD) approach. In this study we extract structure information of vinblastine and some thermodynamic parameters and energy of vinblastine in different temperature from this method. The results indicate good agreement in all of the methods.

COMPUTATIONAL METHOD

Vinblastine model

It was previously demonstrated in studying vinblastine effects on tubulin spiraling, that the catharanthine moiety or the upper half of the vinca alkaloid structure (fig. 1) alone can achieve 70% of the effect of the parent compound [7]. The vindoline moiety alone (lower half) did not induce tubulin spirals, and these authors, therefore, suggested it may be important in anchoring the drug molecule. Important part of the molecule, potentially interacting directly with tubulin side chains via hydrogen bonding [8].



R_1	R_2	R_3	R_4	R_5	[ ]
CH_3	OCH_3	$COCH_3$	CH_2CH_3	OH	-

Fig. 1. Chemical structures of test compound.

Application of Wyman linkage theory to this system demonstrates that the overall drug binding affinity is the product of equilibrium constants, K_1K_2, referred to as the spiraling potential, where K_1 describes drug binding to the tubulin heterodimer, and K_2 is the stepwise interaction of liganded-heterodimers to make an indefinite spiral polymer [9]. Recently, spiraling potential and thermodynamic analysis of the interaction of vinblastine with tubulin has been considered at several temperature 5, 15 and 25^{0}C in presence GTP or GDP [10].

Periodic boundary conditions and solvent

For simulating aqueous system using Hyper chem program, selective salvation of solutes in water is possible. For simulation of big system with constant density, this program uses only rectangular boxes and periodic boundary simulations for core box. Solute float in water well and arrange in a row. Then water molecules, located lesser than determined distance, is eliminated. TIP3P model [11], used by Hyper

chem in periodic box, give atomic charge that produces electrostatic interaction between water molecules carefully. The charge of Oxygen is -0.834 that gives nearest of charge amount rather than amount of electrostatic potential of TIP3P. AMBER calculations use from the information of atomic charge(Fig.2).

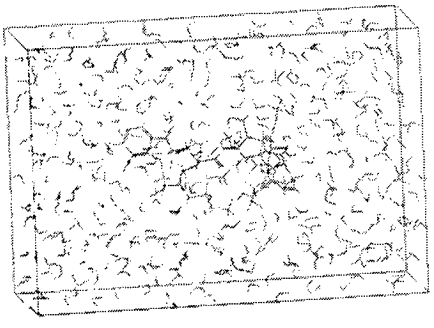


Fig. 2. Molecular structure of water-vinblastine at 1000 ps and 310 K.

Simulating Methodology

Continuum solvation models have been largely exploited in classical simulations,such as molecular dynamics(MD),Monte Carlo(MC) or Langevin dynamics(LD),to reduce computational efforts and to simplify two of the main difficulties of this kind of approach:the correct representation of the damping of the electrostatic interactions between charges of the solute due to polarization of the medium ,and the treatment of the solvent boundary conditions.

In present work, we studied structure and dynamics of vinblastine in water using MD, LD and MC simulations. The most important part of a computerize simulation of a potential function. Mining the models that is used for computation of energy system as a function of structure. In fact a potential function is a mathematical equation that it contains various terms and describes physical interaction. Physical interactions show structures and properties of a system. In this work MC, LD and MD simulation of vinblastine-water complex were performed using Hyperchem and AMBER-94 force field. AMBER (an acronym for Assisted Model Building and Energy Refinement) is a family of force fields for Dynamic behavior of biomolecules. The potential energy is computed by:

$$E_{pot} = \sum_{bond} \mathrm{K}r\left(r - r_0\right)^2 + \sum \mathrm{K}\theta\left(\theta - \theta_0\right)^2 + \sum \frac{vn}{2}\left[1 - \cos\left(n\phi - \phi_0\right)\right] + \sum\left[\frac{\mathrm{A}ij}{Rij^{-12}} - \frac{\mathrm{B}ij}{Rij^{-6}}\right] + \sum \frac{qiqj}{\varepsilon Rij} + \sum_{ij\varepsilon H-bond}\left[\frac{Cij}{Rij^{12}} - \frac{Pij}{Rij^{10}}\right]$$

Where the first term is bonding energy, $r0$ and r are equilibrium bond length and equilibrium length, respectively. The second term is angle energy, $\mathrm{K}r$ and $\mathrm{K}\theta$ are bonding force constant and angle force constant, respectively. The third term is Dihedral angle of energy, Vn, force constant and n is multiplicity or alternative number that shows number of 360^0 rotation cycle around the dihedral. Fourth term shows van der Waals interactions and fifth term is related to electrostatic interactions. This force field is used for polymers and small molecules. This model is useful in investigation of geometric structure of gas phase, free energy of desolvation and vibration frequency of conformation energies. The manner that atoms are shown with it in amber method is monotonic and shows hydrogen atoms in distribution that applies for heavy atom bonded them, and in this way computation in amber force is more rapid rather than other force field. An important feature of amber force field is expression of terms connected to dihedral angles. To express dihedral angle in other force field, it is expressed only central bonding and final atoms are ineffective in potential energy, but in this force field only are used one term for dihedral expansion. Generally, to retain stereochemistry in chiral center, this force field uses dihedral angle between surface of 3 atoms and fourth atom.

Simulation details

Simulation is carried out using periodic boundary conditions. At first geometrical structure of vinblastine that had been geometry optimized using the ab initio molecular orbital calculations with GAUSSIAN 98 program in the gas phase at the Hartree-Fock (HF) with 6-31g basis sets is selected for computational molecular mechanic. Mentioned structure was optimized with amber

molecular mechanic. Then this structure was dissolved in a water box (dimensions of box is 22.6* 18.7* 35.4). Long runs with 451 water molecules. Before running amber program, water-vinblastine complex was optimized too. The TIP3P model was used for water molecules. The collection has constant atom numbers (N), fixed temperature (300^{0}C using temperature bath) (T) and fixed volume (V). Therefore produced collection is a canonical ensemble (V, T, and N). Said system is simulated at four temperatures (305, 310, 315, and 320) in the same way. Considering that spherical cut off must be selected equal or less than half of smallest dimension of box, we used 9.35 and 5.35 A^0 for cut off and (22.6$^\times$ 18.7$^\times$ 35.4) for box. The results recorded in this work are obtained at $1000 ps$ for MC and $100 ps$ for MD and LD methods. Figure 1 shows molecular structure of water-vinblastine at 1000 ps and 310K.

RESULTS AND DISCUSSION

To realize pathway equilibrium with constant temperature simulation, it can be plot variation of kinetic energy and potential energy versus time. When variation is negligible, it is shown that the pathway has equilibrium and simulation has performed successfully. fig.2 shows that the selected pathway is stable.

According to fig.2 can be seen intense fluctuations in initial 20 ps. Intense fluctuations in potential energy release that different parameters of potential energy such as bonding energy, angle bonding energy, dihedral energy, interaction energy, van der Waals, electrostatic and hydrogen bonding change during simulations. It clearly shows hydrogen bonding between vinblastine-water and between water molecules change directions continually, break, form and regulate again. As it is expected total energy equals summation of kinetic and potential energy in any time.

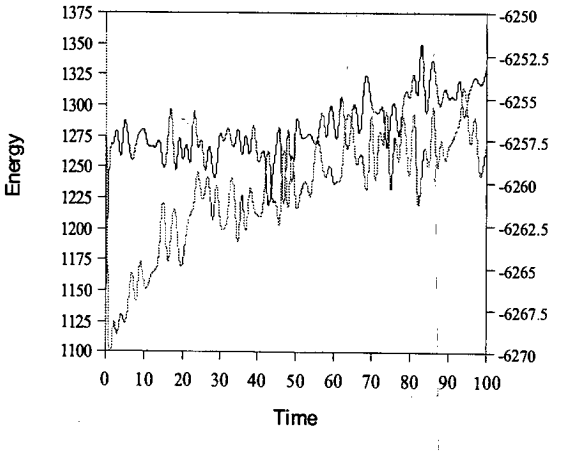


Fig. 3. LD simulation of kinetic energy(bottom) and total energy (top) during 100 ps at 310 K.

The effect of temperature on vinblastine-water complex stability

With absorption of heating energy, motion freedom of atoms increases. With comparing potential energy and total energy of complex, it is seen that when temperature increases from 305 to 315, potential energy does not change. This result is obtained using LD, MD and MC methods. But with LD simulation is shown that with increasing temperature from 315 to 320 K and decreasing temperature from 305 to 300 K, potential energy decreases and therefore complex is more stable (Table 1 and Fig.4).

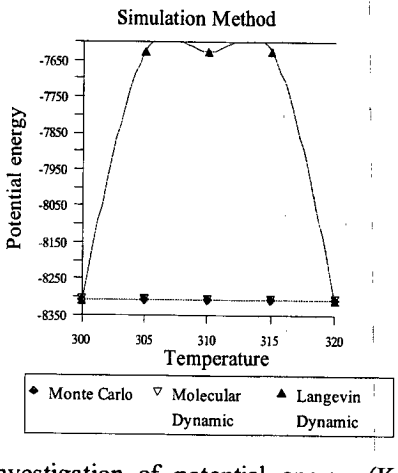


Fig.4. Investigation of potential energy (Kcal.mol^{-1})via temperature(K) at Monte Carlo ,Molecular Dynamic and Langevin Dynamic methods.

Table1. Calculated values of Ekin,Epot and Etot at different simulation force fields and comparison to various temperatures

Temperature(K)	Force Field	Ekin(kCal/mol)	Epot(kCal/mol)	Etot(kCal/mol)
300	MC		-8308.08	
	MD	1314.58	-8308.08	-6993.49
	LD	1314.53	-8308.08	-6993.55
305	MC		-8308.08	
	MD	1336.49	-8308.08	-6971.59
	LD	1336.44	-7627.86	-6291.42
310	MC		-8308.08	
	MD	1358.4	-8308.08	-6949.68
	LD	1358.34	-7627.86	-6269.52
315	MC		-8308.08	
	MD	1380.31	-8308.08	-6927.77
	LD	1380.25	-7627.86	-6247.61
320	MC		-8308.08	
	MD	1402.22	-8308.08	-6905.86
	LD	1402.16	-8308.08	-6905.91

As a result, complex is better in this temperature and stability of system decreases in temperature range at 305 to 315 K. According to these results, LD simulation method for this study is more suitable. The results obtained from LD MD, and MC simulation methods confirm that with decreasing temperature total energy of system decreases(Fig.5a,b and c).

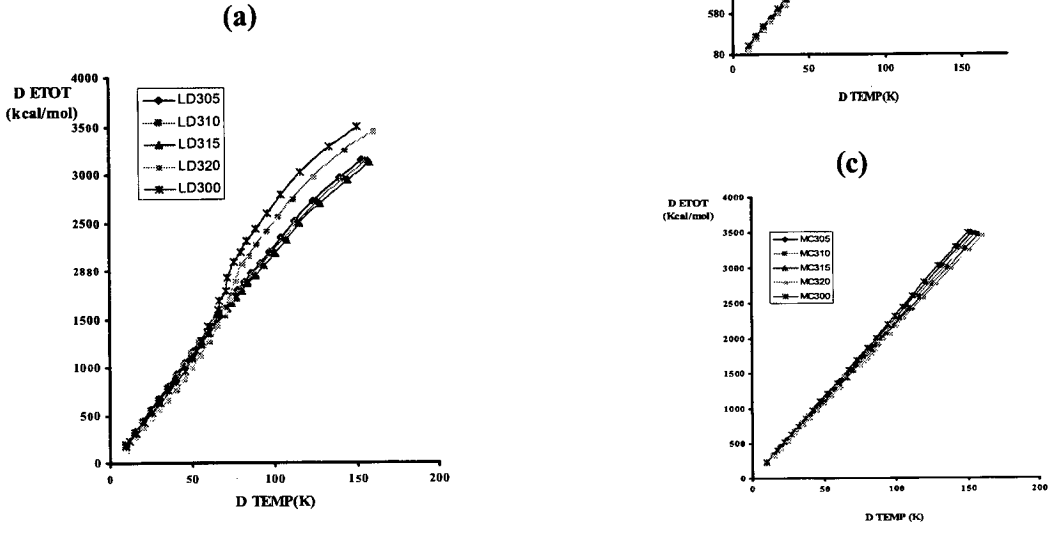


Fig. 5. Effect of temperature on deviation of total energy at three force field (a) langevin dynamic(LD) , (b)(Molecular Dynamic(MD) and (c) Monte Carol (MC) of simulations at 305 to 320 K.

This alterations to be caused by hydrogen bonding, electrostatic and van der Waals energy. Also as it is expected kinetic energy increase with increasing temperature (Fig. 6)

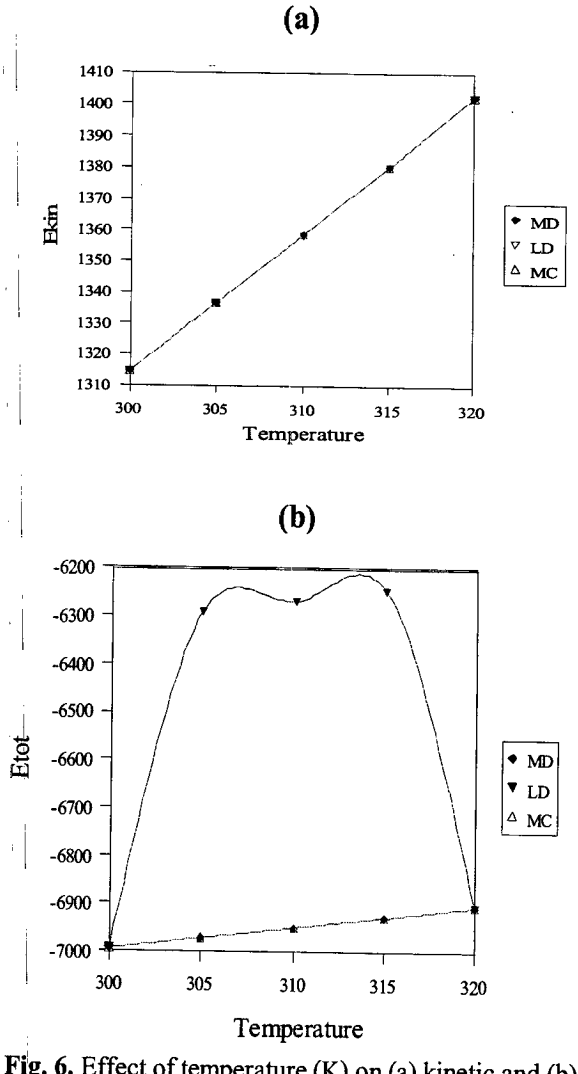


Fig. 6. Effect of temperature (K) on (a) kinetic and (b) total energy (Kcal.mol-1)at three force field of simulations (Molecular Dynamic(MD),Langevin dynamic(LD) and Monte Carol (MC)).

CONCLUSION

The dynamics of microtubules are central to their biological functions. Polymerization dynamics allow microtubules to adopt spatial arrangements that can change rapidly in response to cellular needs and, in some cases, to perform mechanical work. Microtubules utilize the energy of GTP hydrolysis to fuel a unique polymerization mechanism termed dynamic instability. In this review,

we first describe progress toward understanding the mechanism of dynamic instability of pure tubulin and then discuss the function and regulation of microtubule dynamic instability in living cells.

Despite the substantial use of concepts and tools of continuum models in MD/MC/LD simulations we have mentioned so far,only recently have "real" continuum indicated approaches been formulated.

In this paper we present a method which, given a coordinate system, promises to make the subsequent steps both more computationally efficient and simpler, and with solutions interpretable in terms of quantum numbers of characteristic motions where physically possible.

In the following we summarize the method, describing the reasons for the choices, present an application to H2O to energies above dissociation. Finally we look at future applications and improvements and caveats in the approach.

REFERENCES

1. J. J. Correia,and S. Lobert, Current Pharmac eutical Design.7(2001) 1213-1228.
2. Sh.Lobert,B.Vulevic and J. J. Correia, 35, 21(1996) 6807.
3. R. J.Toso, M. A.Jordan,K. W.Farrell,B. Matsumoto, and L. Wilson, Biochemistry. 32 (1993)1285–1293
4. E. Hamel, in Microtubule Proteins ; CRC Press:Inc.Boca Raton.FL(1990)89 –191.
5. R. H. Himes, Pharmacol. & Ther;51(1991) 257–267.
6. S.Rai, J. Wolff, Biol.Chem. 271(1996)14707-14711,
7. V. Prakash, S. N. Timasheff, Biochemistry. 30 (1991) 873-880.
8. S.Lobert, J. Fahy, B. T. Hill, A. Duflos, C. Entievant, J. J. Correia,Biochemistry. 39 (2000) 12053-12062.
9. G.C. Na, S. N. Timasheff, Biochemistry. 19 (1980) 1355-1365.
10. S.Lobert, J.W. Ingram, J.J. Correia, Biophysical Chemistry.126(2007) 50-58.
11. W.L. Jorgensen, J. Ch.Madura, R. W. Impey and M. L. Klein, J. Chem. Phys. 79(1983) 926 .