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Full Length Research Paper

Thermodynamics investigation of Ca^{2+} effect on Troponin-C (TnC): A QM/MM study

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Troponin (Tn) is an essential protein in calcium ion settings of skeletal and heart muscle contractions. It has three subcategories (TnC, TnI, TnT) which are connected to filament actin by tropomyosin. TnC controls the calcium in muscle contraction and is the Ca^{2+}-binding subunit of the troponin complex. ATP facilitates separation of myosin from actin filament, eventually making thick filament to slip off thin filament. This results in short sarcomere. In this study, it is investigated that thermodynamics properties increase concentrations of Ca^{2+} and temperature present in lactic acid. Quantum mechanical and molecular dynamics methods were used to analyze the structure and stability of TnC-Ca^{2+} configuration. TnC-Ca^{2+} mutated active site in lactic acid and water solvent. Calcium ions approaching TnC caused twisting movement of TnC, so TnI-TnT eventually released the head of Myozin to actin. Increased lactic acid can increase calcium ion connection around TnC and decrease Ca to SR; increased temperature can increase metabolism and fatigue of skeletal muscles, emptying glycolysis and reducing muscle activity.

Key words: Troponin C (TnC), calcium ion (Ca^{2+}), lactic acid, fatigue of skeletal muscles, QM/MM.

INTRODUCTION

Muscle contraction is caused by interactions between two major contractile proteins, myosin and actin, which form the thick and thin filaments of muscle, respectively. In the skeletal and cardiac muscles, intracellular Ca^{2+} concentration controlled contraction and troponin; the sole Ca^{2+}-receptive protein in the myofilament plays the key role in the regulation of the sliding action between these two filaments (Raouf and Mahdavian, 2010). Troponin (Tn), with a relative molecular mass of approximately 80 kDa, is composed of three polypeptide chains, TnT, TnI and TnC, and together with tropomyosin (Tm), is located in the polymerized-actin at a Tn:Tm: actin ratio of 1:1:7 (Figure 1) (Gordon et al., 2000).

The Ca^{2+} binds to receptors on muscle that causes rotation and its potential provides action in muscle cells. Ions are linked to troponin-C (TnC-Ca^{2+}) and causes space between them which alters protein-protein interactions in the other thin filament proteins (Raouf and Mahdavian, 2011). For investigation of the direct effects of Ca^{2+} on tropomysin mobility, low levels of Ca^{2+} is determined primarily on thin filament activation (Gonzalez et al., 2005).

TnC binds a total of four Ca^{2+} (Moss et al., 1985); two in low-affinity, Ca-specific sites and two in high affinity sites which can also bind Mg^{2+} (Ojima et al., 2000; Braga et al., 2006). Recently, the x-ray structures of proteins from TnC species crystallized are in the tetragonal system $P2_12_121$ with nearly identical constants cell unit: a =32.34, b = 57.55 and c = 102.13 °A, α=β=γ= 90° (http://www.rcsb.org/pdb). Ball and stick model of interaction and TnC with Ca^{2+} are simulated by Chem3D (Figure 2) and interaction between them is calculated by CHARMM (Brooks et al., 1983) and GAUSSIAN program package (Frisch et al., 1998).

COMPUTATIONAL DETAILS

Density functional theory (DFT) and molecular orbital (MO) calculations were carried out using B3LYP/6-31G*//B3LYP/6-31G* levels of theory with the Gaussian programs package (Frisch et al., 1998). Energy minimum molecular geometries were located

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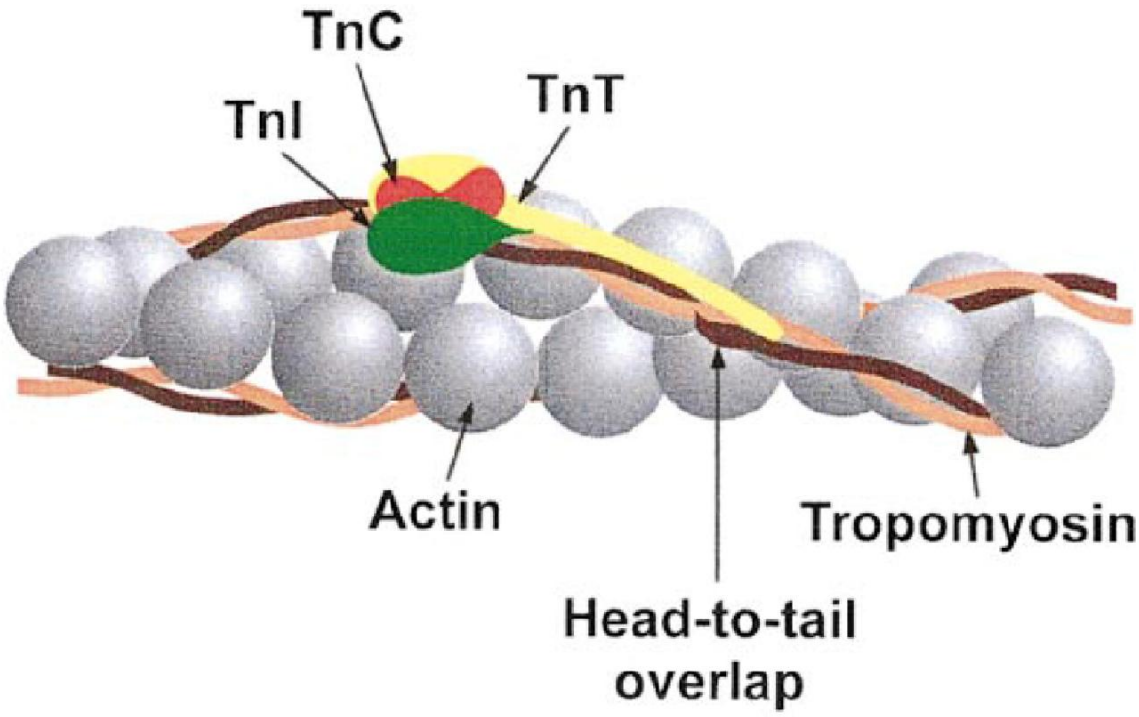

Figure 1. Molecular arrangement of the proteins within the muscle thin filament (Gordon et al., 2000).

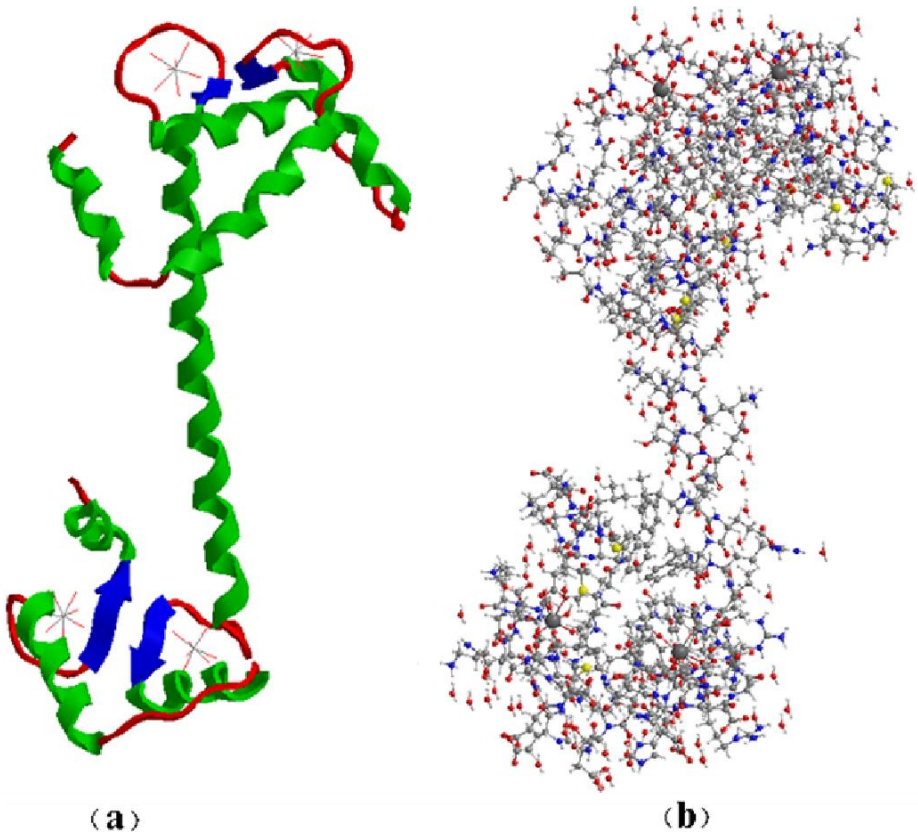

Figure 2. Structure of Troponin-C in muscle contraction: a) View Ribbon Crystal structure TnC-Ca^{2+}, b) Ball and stick model of interaction and TnC configuration rotation with Ca^{2+}.

minimizing energy, with respect to all geometrical coordinates without imposing any symmetrical constraints. NBO analysis was then performed on optimized structures in vacuum and the different solvent media by using B3LYP/6-31G* level of theory by the NBO 3.1 program (Glendening et al., 1995).

Input structures for quantum mechanical calculations were obtained by molecular dynamics simulations utilizing CHARMM (Chemistry at Harvard Molecular Mechanics) program. To model the bulk effects on zwitterionic configuration, we first performed a volume calculation to determine cavity radii required for solvent model in our calculations. The CHARMM program is a general purpose molecular mechanics and molecular dynamics simulation program (Brooks et al., 2009).

The core of the program is based on the empirical potential energy function discussed earlier which is used for energy minimization, molecular dynamics simulations, vibrational analysis and thermodynamic calculations on molecules of biological interest, including proteins, peptides, lipids, nucleic acids, carbohydrates, and small molecule ligands, as they occur in solution, crystals, and membrane environments. In addition, there are interfaces to several quantum mechanical programs which allow one to do mixed QM and MM calculations. The program contains a comprehensive analysis facility which enables the user to compare structures, evaluate energies, and calculate time series and correlation functions.

RESULTS AND DISCUSSION

In present study, the structure of Troponin C was designed in a ball-and-stick model using the data obtained from the X-ray diffraction and NMR spectroscopy as well as Monte Carlo simulation; then, the obtained model was evaluated through changing lactic acid concentration around it and increasing the temperature in time of muscle contraction. Indeed, the empirical data obtained from electromyography (EMG) – the study of muscle function through analysis of the electrical signals produced by the muscle during contraction – have been employed for assessing the aforementioned changes.

In present research, we attempted to describe the kind of atoms and their angles, using the data obtained from protein data bank (pdb), X-ray diffraction, and NMR spectroscopy on the protein crystal structure. The molecular dynamics simulation was carried out with periodic boundary conditions. The zwitterionic structure was solvated at the center of a 33 Å× 33 Å× 33 Å cube of pre-equilibrated water molecules at T=300K and P=1atm. The water molecules for which the oxygen atom was closer than 2.8 Å to any non-hydrogen peptide atom were removed.

The resulting molecular systems contained 2948 atoms, 893 groups and 345 residues for Ca^{2+}-TnC. The water molecules plus peptide system was minimized using the SD (steepest-descent) and ABNR (adopted basis Newton–Raphson) modules of CHARMM to relax the molecular system. The systems were minimized 1500 steps of steepest-descent minimization followed by 1500 steps of adopted basis Newton–Raphson minimization (Jacoby et al., 1972; Wiberg, 1965).

Finally, molecular dynamics simulations of 1000ps at 300 K were performed. All used methods confirmed that the relative stability of Zwitterionic configuration in water and the considered polar solvents lactic acid. The time evolution of the total energy (MJ) of the average structure from explicit solvent simulations relative to the starting structure at t = 0 until t =1000 ps is plotted in Figure 3. Over a 1000 ps of time, with Ca^{2+} approaching NH_2 place, the total energy (E_{total}) is reduced and its structure is stable. Figure 4 showed changing of potential and kinetics energy (MJ) for this interaction.

Calcium binds to the receptors on the muscle and causes a rotation, and then an action potential in the muscle cell. The action potential is then transferred to the sarcoplasmic reticulum through transverse tubules (T-tubules); and through this process the intracellular concentration of Ca^{2+} is raised to 1000 ps. Next, calcium ions bind to C-troponin subunit (sTnC-Ca^{2+}) and creates a conformational change in it.

We have also attempted to calculate the dynamic parameters of the increase of lactic acid molecules on Troponin C in time of contraction (that is, Ca^{2+} binding to its sites). Table 1 summarizes these parameters. Ca^{2+} approaching NH_2 and COOH sites causes a compaction (that is, reduction in the angles between TnC atoms) in the protein and this configuration change yields the energy required for TnI-TnC bond as well as the necessary force for actin rotation by Tm-TnT. As the number of lactic acid molecules around Troponin C is increased, Gibbs free energy, kinetic energy (MJ/mol), bipolar momentum (D) are all increased, but the potential energy measured for this concentration change around TnC shows an initial rise but it is reduced as the number of lactic acid molecules reaches 40% of the weight.

Concentration of hydrogen ion (H) is increased by the rise of lactic acid concentration, then a rise in acidity and consequently a disturbance in enzymatic reaction is observed. This situation results in a delay in release of Ca^{2+} from its site on TnC as well as lengthening of contraction period (that is, spasm). Human body functions within a temperature range of 37 to 39°C. It normally maintains the balance of its internal situation through heat emission. When the temperature is high, the amount of water in the body is reduced due to water loss through perspiration, thus the body ability to adjust its internal temperature is weakened, and it might undergo sunstroke.

Excessive heat and painful tensity in the muscles which appear after severe exercises are the direct consequences of a higher temperature of the body. To elucidate this issue, thermodynamic parameters of TnC were calculated against the temperatures between 36 and 40°C (Table 2). As the temperature is lowered by one degree from 37 to 36°C, kinetic energy (E_{kin}), total energy (E_{total}), and acceptance ratio (ACCR) of the interaction are increased and cause shivering or involuntary movements of the muscles to increase body

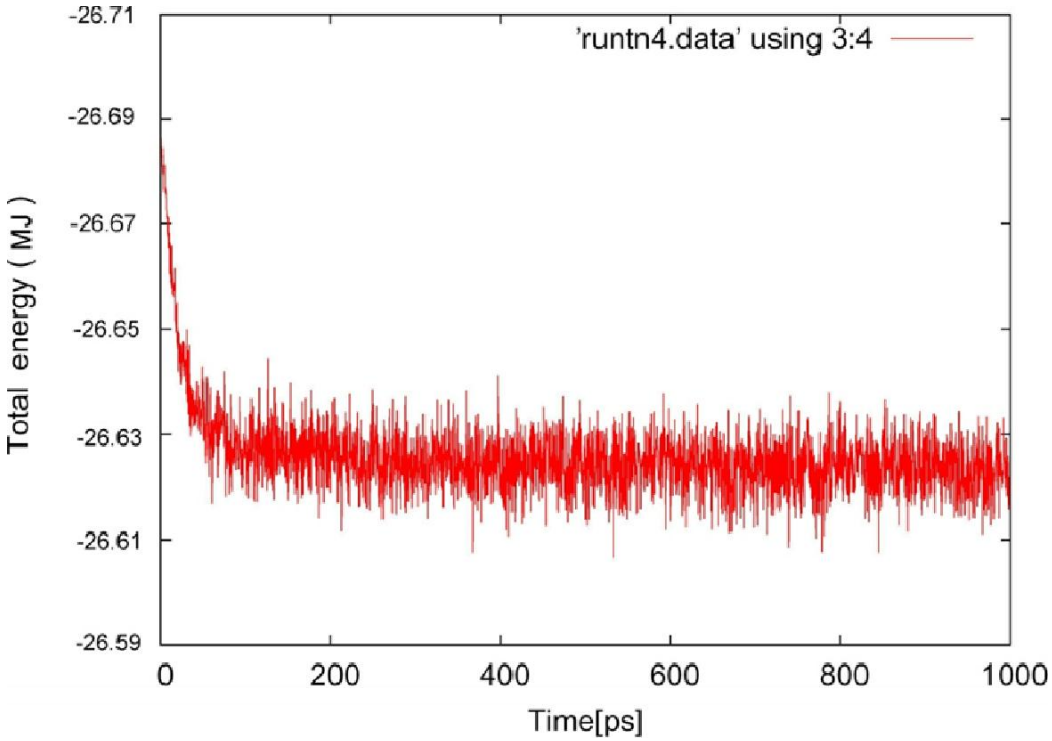

Figure 3. Time history of the overall the total energy (MJ) of the average structures during 1ns=1000ps in water-lactic acid simulation.

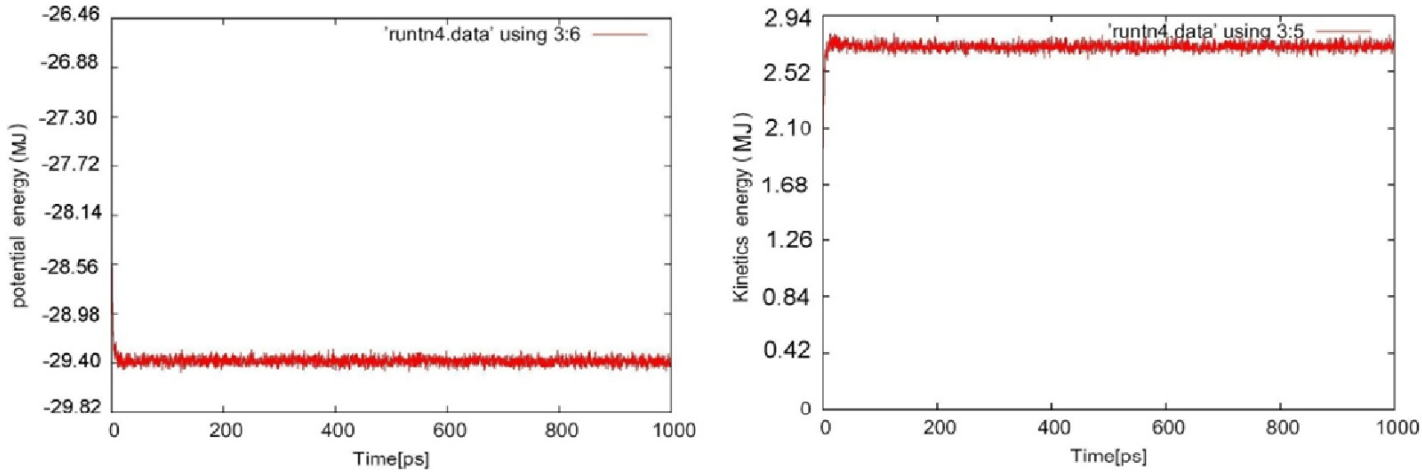

Figure 4. Time history of the overall the potential and kinetics energy (MJ) of the average structures during 1ns=1000ps in water-lactic acid simulation.

heat; similarly, contraction rate and muscle power are considerably increased with a temperature fall. In addition, with a gradual temperature rise in a contracting TnC from 37°C to higher temperatures, the total energy and ACCR are reduced, although kinetic energy is not of a definite trend as the temperature rises.

Overall, the studied structures remain stable, although one can observe a periodic increase and decrease in the root mean square (RMS) values during the simulation time in Figure 5. The obtained results from time series analysis show that the root mean square deviation (RMSD) and the radius of gyration (Rg) average values for Ca^{2+}-TnC are 1.65 and 22.54 whose center of mass was X=-2.45382, Y=42.64709, Z=14.07996 and time step was 4.09×10^{-02}.

As we know, the CHARMM force field uses flexible

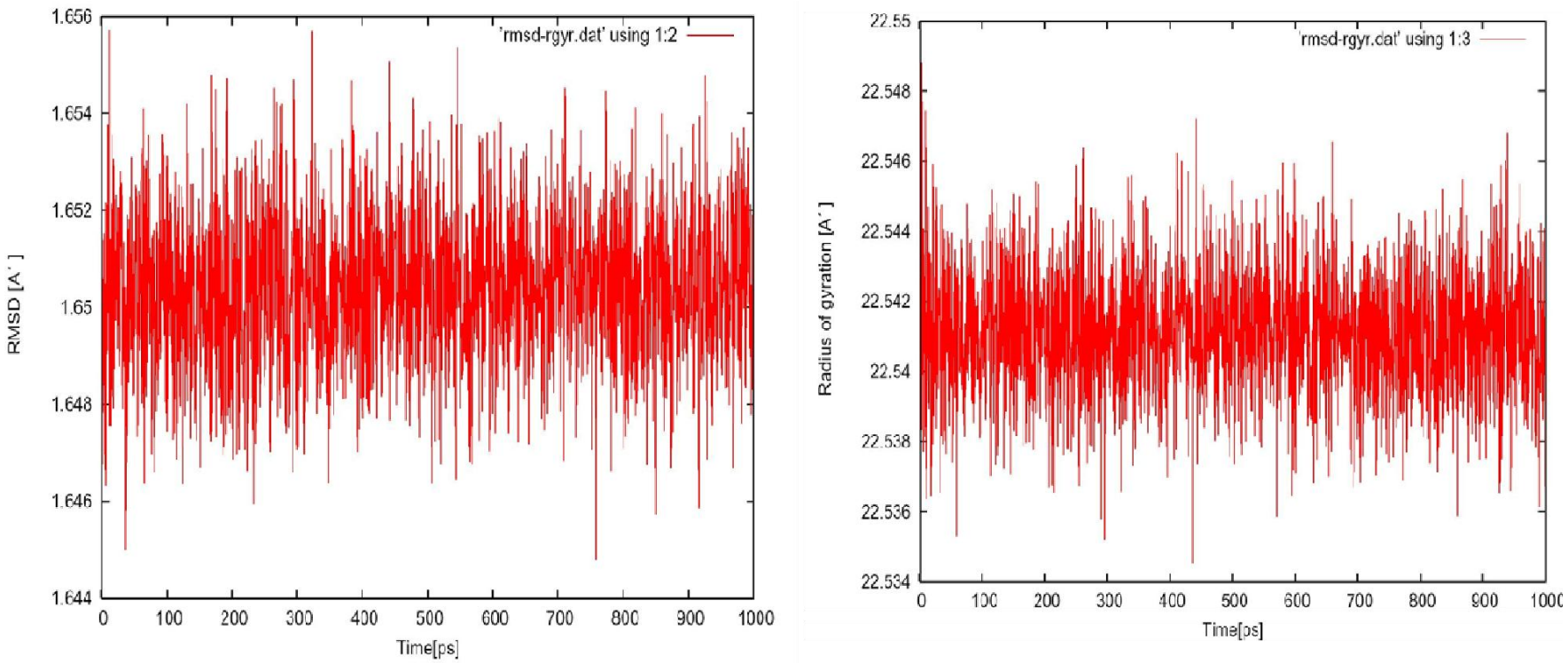

Figure 5. Time history of the overall RMS deviations (RMSD) and radius of gyration (Rg) of the average structures from the starting structures during 1 ns explicit water-lactic acid simulation.

Table 1. Thermodynamics parameters of increasing the number of lactic acid molecules around TnC.

Wt% lactic acid	G_{ele} (MJ/mol)	E_{kin} (MJ/mo l)	E_{pot} (MJ/mol)	E_{ele}(v)	ACCR	Dipole moment (D)	RMS (MJ/mol·Å)
10	786.50	11.43	-158.80	-8150.26	0.019	2101	8.54
20	1013.83	11.77	-145.87	-10506.05	0.017	2184	8.44
30	1054.02	12.11	-265.05	-10922.52	0.419	2289	8.56
40	7004.86	12.45	-206.69	-72589.27	0.419	2362	27.24

(G_{ele} = electrical Gibbs free energy, E_{kin} = kinetics energy, E_{pot}= potential energy, E_{ele}= Electrical energy, ACCR= acceptance ratio, RMS = root mean square).

bond, angle bends, dihedral bond torsions, improper dihedral bond torsions, Lennard/Jones terms for non-bonded interactions and 1- 4 interactions, electrostatic interactions between partial charges located at the atoms and Urey/Bradley (UB) terms for 1-3 interactions. The

Table 2. Thermodynamics parameters of temperature rise of TnC in contraction mode.

θ(^{O}C)	E_{total} (MJ/mo l)	E_{kin} (MJ/mo l)	E_{pot} (MJ/mo l)	ACCR
36	749.04	11.50	738.54	0.93
37	519.12	11.37	507.75	0.48
38	517.09	11.40	505.69	0.47
39	512.59	11.36	501.22	0.47
40	523.56	11.47	512.09	0.48

(E_{total} = Total energy, E_{kin} = kinetics energy, E_{pot}= potential energy, ACCR= acceptance ratio).

general form of the potential is given by Equation 1 (Brooks et al., 2009):

$$U(R) = \sum_{bonds} K_b (b - b_0)^2 + \sum_{angles} K\theta (\theta - \theta_0)^2 + \sum_{UB} K_{UB} (S - S_0)^2 + \sum_{dihedrals} K_\phi (1 + Cos(n\phi - \delta)) + \sum_{impropers} K_\omega (\omega - \omega_0)^2 + \sum_{non\text{-}bonded\ PAIRS} \varepsilon_{ij}^{MIN} \left[\frac{R_{ij}^{MIN}}{r_{ij}} \right]^{12} - 2 \left[\frac{R_{ij}^{MIN}}{r_{ij}} \right]^{6} + \frac{q_i q_j}{4\pi\varepsilon_o r_{ij}} + \sum_{residues} CMAP(\phi\psi) \quad (1)$$

The potential energy, U (R), is a sum over individual terms representing the internal and non-bonded contributions as a function of the atomic coordinates. Internal terms include bond (b), valence angle (θ), Urey– Bradley (UB, S), dihedral angle (ϕ), improper angle (ω), and backbone torsion correction ($CMAP(\phi,\psi)$) contributions as shown in Equation 1. The parameters K_b, K_ϕ, K_{UB}, K_θ and K_ω are the respective force constants and the variables with the subscript 0 are the respective equilibrium values. All the internal terms are taken to be harmonic, except the dihedral angle term, which is a sinusoidal expression; here n is the multiplicity or periodicity of the dihedral angle and δ is the phase shift. The all-atom implementations of the CHARMM force field include all possible valence and dihedral angles for bonded atoms, and the dihedral angle term about a given bond may be expanded in a Fourier series of up to six terms (Brooks et al., 2009).

Conclusion

In this work, we have investigated the implicit and explicit effects of the surrounding water molecules (the bulk water-lactic acid effects), the dielectric constant effects and the effects of side chain mutation in point of view of atomic characterizations by quantum mechanics calculations and molecular mechanics simulations on the considered active site. The obtained results provide a reasonable picture from structural and energetic proteins. This research can help us to understand solvation and mutation processes at a molecular level in TnC.

To obtain reliable results regarding muscle contraction, the smallest part of muscle (that is, Troponin C) located on actin filament was assessed through simulated computations. With Ca^{2+} approach to Troponin C, the configuration of the protein and all the angles between its amino acids are changed which causes a movement in different parts of troponin. In the present study, we conducted a thermodynamic and kinetic investigation on Ca^{2+} approach to NH_2 sites located in Troponin C. The results obtained here indicate that the interaction is exothermic and yields a high amount of potential energy for TnI displacement.

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