Int. J. Ind. Chem., Vol. 1, No. 1, 2010, pp. 39-45

International Journal of Industrial Chemistry

www.ijichem.org

Islamic Azad University
Quchan Branch

ISSN (online): 2228-5547
ISSN (print): 2228-5970

Density Functional Theory of Ca^{2+} and Troponin C (TnC)

Mahmoud Raouf[a] , Leila Mahdavian[b]*

[a]*Department of Physical Education, Islamic Azad University, Doroud Branch, P.O. Box: 133, Doroud. Iran.*
[b]*Department of Chemistry, Islamic Azad University, Doroud Branch, P.O. Box: 133, Doroud. Iran*
*Email: Mahdavian_leila@yahoo.com

Received: 28 August 2010; Accepted: 25 September 2010

Abstract

In this research, the effects of Ca^{2+} on TnC (Troponin C) are investigated. TnC controls the calcium in muscle contraction and is the Ca^{2+}-binding subunit of the troponin complex. The nanosecond mobility domain presents in solution, as determined by QM/MM of Charmm (Chemistry at Harvard Molecular Mechanics) program, is fully calculated in fibers. The orientation analysis revealed a bimodal orientation distribution of TnC in the presence and absence Ca^{2+}. Thermodynamic properties are calculated for this orientation (TnC and Ca^{2+}) and the Ca2+ interaction with NH_2 and COOH terminals of troponin C, the impact of lactic acid concentration on Ca^{2+} from its sites on troponin C are studied, and the effect of temperature increase on the interaction.

Keywords: Ca^{2+} ion; Lactic acid; QM/MM methods; TnC (Troponin C)

1. Introduction

The thin filament of vertebrate striated muscle is composed of actin, tropomyosin, and troponin (Tn). Troponin is a complex of three proteins:

1. Troponin C (TnC) which the Ca^{2+}-binding component.
2. Troponin T (TnT) which the tropomyosin binding subunit.
3. Troponin I (TnI) which the inhibitory subunit, capable of binding to actin inhibiting actomyosin ATPase activity [1].

The Ca^{2+} binds to receptors on muscle that cause rotation and potential provide of action in muscle cells. Ions link to troponin-C (TnC-Ca^{2+}) and this link causes change in the place of protein-protein interactions and in the other thin filament proteins. For investigation of the direct effects of Ca^{2+} on tropomysin mobility, low levels of Ca^{2+} is determined primarily on thin filament activation [2].

As Fig. 1 shows TnC binds with four Ca^{2+}; two in low-affinity (Ca-specific sites) and two in high affinity (sites which can also bind Mg^{2+}) [3-5]. Recent studies show the X-ray structures of proteins from TnC crystal species are in the tetragonal system $P2_12_121$ with nearly identical unit cell constants: a =32.34, b = 57.55 and c = 102.13 Å , $\alpha=\beta=\gamma= 90^o$ [6]. Ball and stick model of interaction and TnC with Ca^{2+} is simulated by Chem3D (Fig. 2) and interaction between them is calculated by

Archive of SID
www.SID.ir

Charmm [7] and Gaussian program package [8].

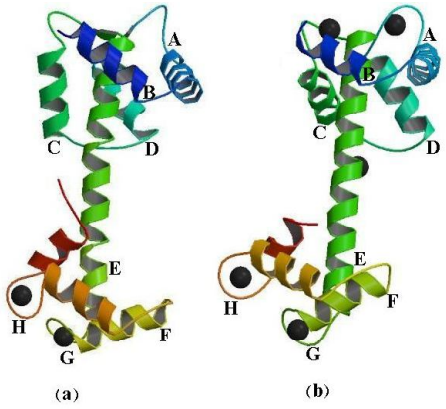

Fig. 1. a) Ribbon crystal structure TnC with Ca^{2+} (black butter) that are linked to two ends COOH and NH_2-TnC is free, b) ribbon crystal structure of TnC binds a total of four Ca^{2+} in two NH_2 and two COOH, calcium ion approached to TnC (rotation can be seen).

Using computational methods, the impact of lactic acid and temperature on troponin C during muscle contraction is studied in this research and the results indicate that accumulation of lactate and H^+ in the exhausted muscle causes a decrease in the return force of Ca^{2+} from NH_2 and COOH sites on troponin C. The calculations were conducted using Gaussian and Charmm software's with semi-empirical methods, together with Monte Carlo simulation.

2. Experimental

As can be seen in Figs. 1 and 2, the simulation of TnC and Ca^{2+} are done using ChemDraw and Chem3D (based on the biochemical data of protein data bank [6]). In Fig. 2, four calcium ions are place in the proximity of COOH functional group; in addition the order of amino acids consisting TnC with curled ribbons is shown (in Fig. 2, the order of amino acids is also presented in ball and stick model).

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 [8]. The minimum energy of molecular structures was located to minima the 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, using B3LYP/6-31G* level of theory by the NBO 3.1 program [9]. Input structures for quantum mechanical calculations were obtained by molecular dynamics simulations utilizing Charmm program. To model the bulk effects on zwitterionic configuration, at the first, we performed volume calculations 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 [10].

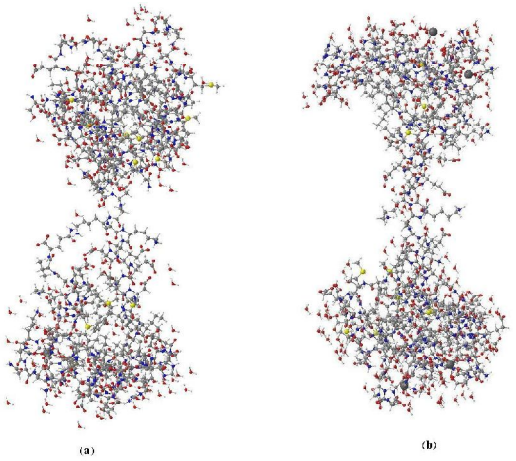

Fig. 2. Ball and stick model interactions and TnC configuration of Ca^{2+} ions approaching.

As we know, the Charmm force field uses flexible 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 general form of the potential is as bellow:

Archive of SID

www.SID.ir

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

The potential energy, U ($\vec{R}$), is a sum of 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 ($CMAP(\varphi,\psi)$) contributions, as shown in eq.(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 [10].

3. Results and Discussion

In this study, the structure of troponin C was designed in a ball-and-stick model using the data obtained from 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– was employed to assess the aforementioned changes.

An important fact is that there are four Ca^{2+} reception sites on protein TnC, and these sites are located at two ends of the protein (COOH and NH_2 ends); the sites (as shown in Fig. 2) are connected together by a longer helix of other amino acids (E), and as Ca^{2+} gets closer to the protein, COOH shows more tendency to interact with Ca^{2+}, in comparison with NH_2 (Fig. 1a). After Ca^{2+} binds with the sites, the two NH_2 sites show more tendency than before to interact with Ca^{2+} [11]. Regarding TnC-TnI connection and the total displacement of actin Some propositions are as follows: a) Sykes et al. [12] suggest that TnC-TnI connection in heart is because of unwrapped position of the NH_2 site on TnC, and the empirical results of Putkey et al. [13] obtained from Fluorescence Resonance Energy Transfer (FRET) verify this hypothesis; b) Cheung et al. [14] noted that TnC is not unwrapped but packed after interacting with Ca^{2+}, and a bond is created between the two proteins. The difference between these two hypotheses originates from the difference between heart and skeletal muscles. Our computational results, on skeletal muscles, indicates that after Ca^{2+} gets closer to the NH_2 sites, it causes a compaction or a reduction in the angles between TnC atoms (Figs. 1 and 2). Such configurationally change provides the energy required for TnI-TnC connection and the necessary force for actin rotation in skeletal muscles by Tm-TnT.

The molecular dynamics simulation was carried out with periodic boundary conditions. The zwitterionic structure was solvated at the center of a 33Å× 33Å× 33Å cube of pre-equilibrated water molecules at T=300K and P=1atm. Water molecules, for which the oxygen atom was closer than 2.8Å to any non-hydrogen peptide atom, were removed. The resulting molecular systems contained 2948 atoms, 893 groups and 345 Å residues for Ca^{2+}-TnC. The water molecules plus peptide system were minimized using the SD (steepest-descent) and ABNR (adopted basis Newton–Raphson) modules of Charmm to relax the

Archive of SID

www.SID.ir

molecular system. The systems were minimized 1500 steps of steepest-descent minimization followed by 1500 steps of adopted basis Newton–Raphson minimization [11, 12]. Finally, molecular dynamics simulations' calculations were performed at 1000ps and 300 K. All results confirm that our considered method can be used to calculate both relative stability of zwitterionic configuration in water and polar solvents of lactic acid. As Fig. 3 shows the time evaluation for total energy (kcal) of the average structure of Ca^{2+}-TnC solvent simulation is performed. Over a 1000 ps, by approaching Ca^{2+} to NH_2 place, the E_{total} reduces and its structure becomes stable.

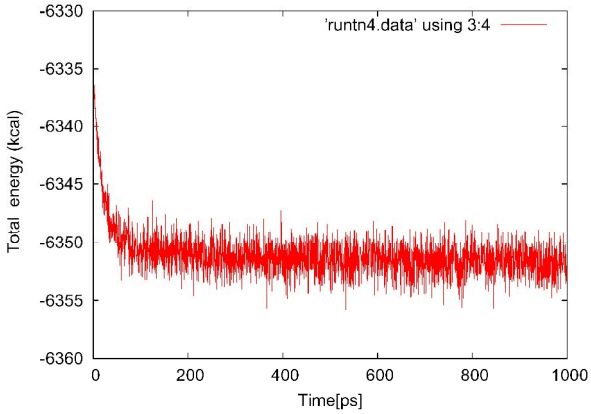

Fig. 3. Time record of total energy (kcal) for the average structure of Ca^{2+}-TnC in "water-lactic acid" simulation (during 1ns).

Table.1 shows the results obtained from the computational methods for Ca^{2+} approaching to NH_2 terminal sites through 5 stages. The calculations of thermodynamic parameters for the first stage are in the absence of Ca^{2+} in NH_2 site. In the next stages, Ca^{2+} gets closer to NH_2 sites through 4 stages until NH_2 and COOH sites are occupied by four Ca^{2+} in the last stage.

Accepted Rate (ACCR) calculated for this interaction (Table 1) shows an increase, which indicates a configuration change and displacement of all atoms of the protein. RMS gradient (kcal/mol/ Å) obtained was lower than 1, which is acceptable for protein compounds, and it was of very slight (negligible) difference for each stage.

Fig. 4 shows chemical potential difference (kcal/mol) for the stages, through which a severe fall is observed in chemical potential difference as the number of calcium ions are increased around TnC, and they approach NH_2 sites of troponin C. Occurrence of a potential wave in the early stages (Fig. 4, a-b) indicates Ca^{2+} presence in TnC where Ca^{2+} causes a sudden rotation and change in TnC structure; then as Ca^{2+} moves to its binding site (i.e. NH_2 sites), a continuation in negative trend of potential difference is observable (Fig. 4, c-d). It is also worth mentioning that the negative potential difference in the stages illustrate the automatic and exothermic nature of the mentioned interaction.

Table 1. The computational methods of thermodynamics parameters of calcium ion approaching to NH_2-TnC.

Steps	Langvian Dynamic Simulation			Monte Carlo Simulation			
	E_{total} (kcal/mol)	E_{pot} (kcal/mol)	Dipole moment (D)	E_{total} (kcal/mol)	E_{pot} (kcal/mol)	ACCR	RMS kcal/mol·Å
1	11874.54	5604.33	30.19	11463.38	8756.86	0.093	0.859
2	10839.08	5146.67	29.48	11288.18	8580.73	0.119	0.858
3	10810.87	5120.02	28.86	11189.95	8482.50	0.157	0.857
4	10725.47	4858.69	28.28	11088.18	8380.73	0.175	0.856
5	10691.38	4999.01	27.52	10821.18	8114.11	0.198	0.855

www.SID.ir

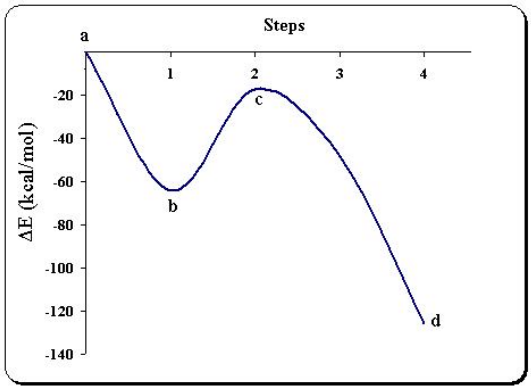

Fig. 4. Chemical potential difference of Ca^{2+} in place NH_2-TnC.

Fig. 5 shows changing of potential and kinetic energy (kcal) for this interaction. Calcium binds with the receptors on the muscle and causes a rotation, and then a sudden action in the muscle cell. The action is then transferred to the sarcoplasmic reticulum through transverse tubules (T-tubules); and through this process the intracellular concentration of Ca^{2+} is raised until 1000 ps. Next, calcium ions bind to C-troponin subunit ($TnC\text{-}Ca^{2+}$) and create a conformational change in it.

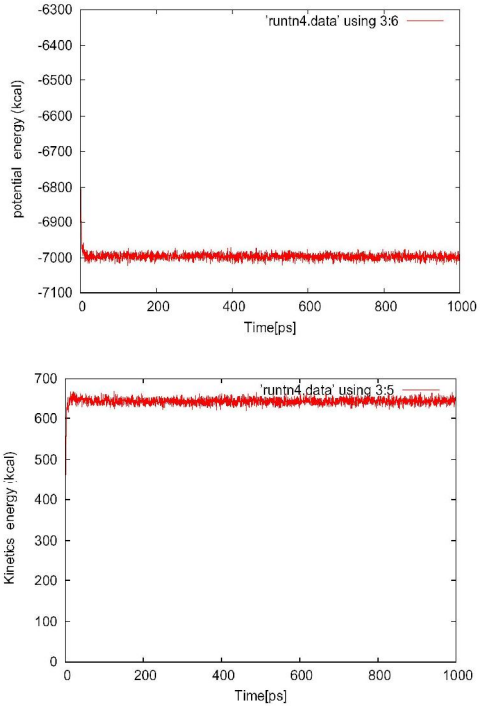

Fig. 5. Time record of potential and kinetic energy (kcal) of the average structures in "water-lactic acid" simulation (during 1ns).

We have also attempted to calculate the dynamic parameters of the increase of lactic acid molecules on troponin C during contraction (i.e. Ca^{2+} binding to its sites); Table 2 summarizes these parameters. Ca^{2+} approaching to NH_2 and COOH sites causes a compaction (i.e. 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 their weight.

Our 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, so the body ability to adjust its internal temperature become weak, 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 3). As the temperature was lowered as one degree from 37°C to 36°C, kinetic energy, total energy, and ACCR of the interaction increased and caused shivering or involuntary movements of the muscles to increase body heat; similarly, contraction rate and muscle power 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 reduced, although kinetic energy is not of a definite trend as the temperature rises.

Archive of SID
www.SID.ir

Fig. 6 presents the impacts of temperature rise on potential energy. Furthermore, temperature rise causes an increase in the amount of oxygen consumption which leads to higher glycogen consumption and consequently higher concentration of lactic acid in the muscles, is a factor involved in fatigue occurrence.

4. Conclusions

According to the previous physiological and biochemical studies on providing the energy required for troponin-tropomyosin slide and actin rotation for binding to the myosin head, there is no clear answer for this issue, since there are many limitations in both experimental and field studies; thus preparing a virtual lab to achieve the simulated muscle situation, inspired us to conduct such calculations using chemical and biochemical softwares.

To obtain reliable results, regarding muscle contraction, the smallest part of muscle (i.e. troponin C) located on actin filament was assessed through simulated computations. With Ca^{2+} approaching to troponin C, the configuration of the protein and all the angles between its amino acids changes which causes a movement in different parts of troponin. In this study, we conducted a thermodynamic and kinetic investigation on Ca^{2+} approaching to NH_2 sites located on troponin C and investigated the increase in lactic acid amount as well as temperature on activity and configuration change of troponin C. The obtained results indicate that the interaction is exothermic and yields a high amount of potential energy for TnI displacement.

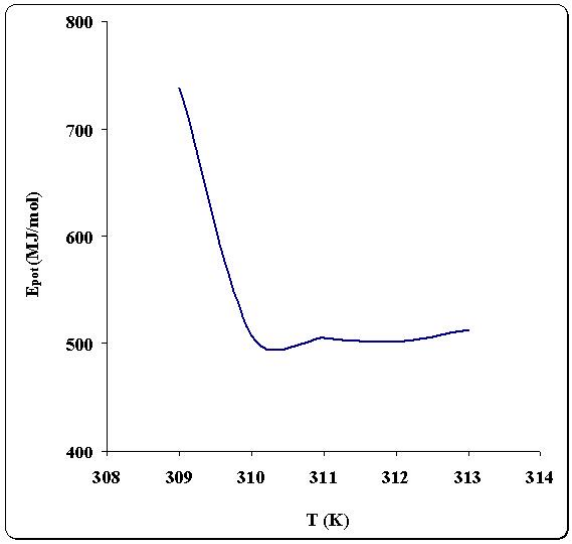

Fig. 6. temperature increase on the potential energy (MJ/mol).

Table 2. Thermodynamics parameters of increasing the number of lactic acid molecules around TnC.

Wt% lactic acid	G_{ele} MJ/mol	E_{kin} MJ/mol	E_{pot} MJ/mol	E_{ele} (v)	ACCR	Dipole moment (D)	RMS MJ/molÅ
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

Table 3. Thermodynamics parameters of temperature rise of TnC in contraction mode.

θ(°C)	E_{totel} MJ/mol	E_{kin} MJ/mol	E_{pot} MJ/mol	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

Archive of

www.SID.ir

References

[1] H. Ch. Li and P.G. Fajer, Biochemistry 33 (1994) 14324.
[2] A. M. Gonzalez, J. Fredlund, M. Regnier, J Physicol 562 (2005) 873.
[3] R. L. Moss, G. G. Giulian, M. L. Greaser, J Gen Physiol 86 (1985) 585.
[4] T. Ojima, N. Koizumi, K. Ueyama, A. Inoue, K. Nishita, J Biochem 128 (2000) 803.
[5] C. A. C. A. Braga, J. R. Pinto, A. P. Valente, J. L. Silva, M. M. Sorenson, D. Foguel, M. C. Suarez, Int J Biochem Cell B 38 (2006) 110.
[6] http://www.rcsb.org/pdb
[7] B. R. Brooks, R.E. Bruccoleri, B. D. Olafson, D.J. States, S. Swaminathan, M. Karplus, J Comput Chem 4 (1983) 187.
[8] M. J. Frisch et al., Gaussian, Inc., Pittsburgh PA, 1998.
[9] E. D. Glendening, A. E. Reed, J. E. Carpenter, F. Reinhold, NBO Version 3.1.
[10] B. R. Brooks, C. L. Brooks, A. D. Mackerell, L. Nilsson, R. J. Petrella, B. Roux, Y. Won, G. Archontis, C. Bartels, S. Boresch, A. Caflisch, L. Caves, Q. Cui, A. R. Dinner, M. Feig, S. Fischer, J. Gao, M. Hodoscek, W. Im, K. Kuczera, T. Lazaridis, J. Ma, V. Ovchinnikov, E. Paci, R. W. Pastor, C. B. Post, J. Z. Pu, M. Schaefer, B. Tidor, R. M. Venable, H. L. Woodcock, X. Wu, W. Yang, D. M. York, M. Karplus, J Comput Chem 30 (2009) 1545.
[11] J. D. Potter, J. Gergely, Biochemistry 13 (1974) 2697.
[12] M. X. Li, L. Spyracopoulos, B. D. Sykes, Biochemistry 38 (1999) 8289.
[13] D. Keller, Biophysics J 74 (1998) 2743.
[14] Wen-Ji Dong, Jun Xing, Yexin Ouyan, Jianli An, and Herbert C. Cheung, J Bio Chem 283 (2008) 3424.

Archive of SID

www.SID.ir

Surf and download all data from SID.ir: www.SID.ir

Translate via STRS.ir: www.STRS.ir

Follow our scientific posts via our Blog: www.sid.ir/blog

Use our educational service (Courses, Workshops, Videos and etc.) via Workshop: www.sid.ir/workshop