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Theoretical Study of Lead Ions into Troponin C (sTnC) in Muscle Contraction: MM

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ABSTRACT

In recent years, many of human diseases are caused by the arrival of heavy metal ions such as lead ions to the human body in different ways. Among of the diseases of Pb^{2+}aremuscle pain, muscle relaxation and etc. Muscle contraction is a result of actin and myosin protein sliding on each other. Rate and mechanism of Ca^{2+} exchange in troponin C in the skeletal muscle are the key point for understanding this interaction.

In this study, we have investigated Pb^{2+} interactions with NH_2 and COOH terminals of troponin C (sTnC) on actin filaments in skeletal muscle. The replacement of lead ions instead of calcium ions in the position famine and carboxyl, is optimized by B3LYP basis set 6-31G.The thermodynamic parameters are calculated using the molecular mechanics (MM) force field of MM^{+}, Amber and BIO^{+}. In addition to, have investigated the changes in concentration lactic acid molecules around the stones. Also the closed lead ions to amine and carboxyl terminals are studied in different distances.

The results shown, at first, calcium ions are located in the amine position and then carboxyl positions are occupied and the lead ions are replaced by calcium ions in the position of amine. For this replacement plenty of energy is necessary which, shows the disruptive energy of lead in troponin C is higher than calcium ions. The total energy shows a significant increase from the rising of temperature and concentration of lactic acid and back muscles expand to the initial state is very difficult and cause muscle pain.

KEYWORDS: Troponin C of skeletal muscle (sTnC), Effects of Lead ions, Thermodynamics properties, Molecular mechanics (MM), Lactic acid.

INTRODUCTION

Heavy metals are the most dangerous pollutants of the surroundings. One of these pollutants is lead. It is spreading in the surroundings and causes for respiratory and neural problems, insomnia, stomachache, schizophrenia, shortage of calcium and muscle aches and so on. The main of entering lead to the body is respiration and the digestion [1,2]. Organic compounds of lead, such as acetate and $Pb(C_2H_5)_4$are absorbed through the skin very well: but mineral compounds firstly spread and deposit in soft tissues such as brain, liver and muscles and then gradually is stored in bony tissues, teeth and hair. Lead can link to the most of necessary compounds of the body, like enzymes and proteins causing interruption of enzyme activity and impairment in protein synthesis [3,4].

One of the effects of entering lead ion into the human body is muscle ache and muscle loose that so far there is no comprehensive mechanism for these reactions. The many studies are done, about preparing an energy for troponin–tropomyosin slip and at the last act of turning to link to the musing head of muscles. But they can't provide a clear and convincing response to this discussion, because there are a lot of limitations in laboratory musculature. It persuaded us to do considerable calculations by the available softwares in chemistry and biochemistry contexts. We can study the reaction of troponin C during muscle contractions as a result, fatigue after they are adjacent to lead ion, then compare them with the resulted physiological data in medical sciences [5]. This project`s goal is to study chemical and physiological reactions of lead ion and troponin in muscle filaments.

In this study, we try to simulate troponin C by the date of X-ray diffraction and NMR spectrometer. The picture of sphere and coil model or ribbon form during absorbing lead ion is shown in figure 1[6]. According to the resulted data is analyzing the data. There are a lot of algorithms for calculations, but regarding choosing the environment around sTnC, water and lactic acid are considered.

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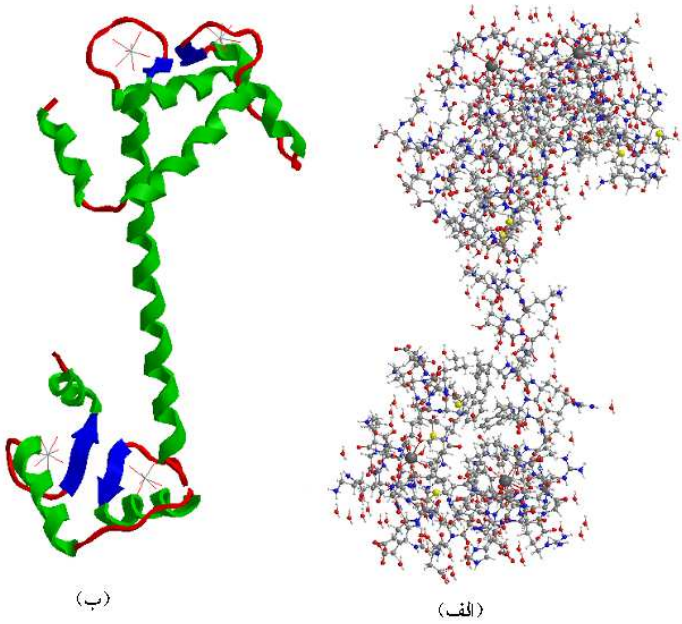

Figure 1: a) Ball and stick model, b) View ribbon crystal structure of sTnC with four ions of Pb^{2+} in NH_2 and COOH of sTnC.

After improvement of structures in each step, thermodynamic parameters are evaluated by Gaussians software (ab initio methods and also mechanic molecule method(MM) such as MM^+, Amber, BIO^+(Charmm) [7-10].

Computational method

After simulation of troponin C, its structure is optimized by Gaussian program package under Linux by 48 core supercomputers with basis set of B3LYP/6-31G. Due to the huge mass of the structure of sTnC with lead ions. The thermodynamic properties of lead ions approaching in amin and carboxyl places in sTnC were calculated molecular mechanics methods [11, 12] on the basis of MM^+, Amber and BIO^+(Charmm). The force fields of molecular mechanics power are used for classic equations and describe the surfaces of potential energy and physical features of molecules [13]:

A: molecule is series of atoms and reactions, which is described by simple analytical functions. These descriptions are called power field. Compounds energy of power field comes from links density and traction. Molecular mechanic fields, including harmonious terms and are limited to computation of diametrical matrix elements of power constant.

B: power fields, including the terms of higher square, for example 3, 4 and the terms computing the elements of non- diametrical matrix of power constant.

C: the third group of the power fields includes classic terms and effects of electronegativity or super coupled.

Although power fields MM^+, Amber and BIO^+(Charmm) were used for a limited number of compounds but today are used for an extensive domain of proteins, nucleic acid, nano-surfaces, and are well known, precise are necessary.

DISCUSSIONS AND RESULTS

The muscle contractions are the result of linkage between actin protein and the head of myosin, the rotation due to exposure to calcium ions in amine and carboxyl places of sTnC. When muscles are at rest, calcium ions are stored in the sarcoplasmic reticulum (SR). The muscle is stimulated sufficient by nerve impulses; Ca^{2+} ions are released from SR and combine with troponin C (TnC).When action potentials stop arrives, calcium is actively pumped back into the SR, tropomysion blocks the myosin head binding sites on the actin, and the muscle relaxes. When lead ions enter the body in different ways, they go around the muscles and replace the calcium ions. In this study is simulated and computed the approaching steps of lead ion in four places in troponin C of skeletal muscle.

- **Entry lead ions into sTnC**

The thermodynamic properties of replaced lead ions to calcium ions are shown in table 1.The four places in troponin Care occupied by calcium ions. In the first step, lead ion is replaced in calcium of amine places, then carboxyl places which are computed by three methods of molecular mechanics.

Table 1: Thermodynamics parameters of calcium ions in NH_2& COOH-sTnC positions.

Status	MM^+			Amber			BIO^+(Charmm)		
	E_{total}mJ/mol	Dipole moment (D)	RMS kcal/mol·Å	E_{total}mJ/mol	Dipole moment (D)	RMS kcal/mol·Å	E_{total}mJ/mol	Dipole moment (D)	RMS kcal/mol·Å
$4Ca^{2+}$	919.62	221.4	624.3	814.11	592.4	802.4	816.60	367.6	787.9
	Lead ions approaching preference to the NH_2 position								
$1Pb^{2+}(NH_2)$-$3Ca^{2+}$	920.42	221.4	624.3	815.50	592	802.4	817.98	367.6	788.0
$2Pb^{2+}(NH_2)$-$2Ca^{2+}$(COOH)	921.29	221.4	624.4	814.49	559.3	802.4	819.41	366.4	788.0
$3Pb^{2+}$-$1Ca^{2+}$(COOH)	922.19	221.4	624.5	815.89	557.2	802.4	820.81	365.6	788.1
	Lead ions approaching preference to the COOH position								
$1Pb^{2+}$(COOH)-$3Ca^{2+}$	931.99	221.4	628.8	816.28	564.1	802.3	821.26	372.3	788.0
$2Pb^{2+}$(COOH)-$2Ca^{2+}(NH_2)$	921.56	221.4	624.5	814.58	557.5	802.4	819.49	367.8	788.0
$3Pb^{2+}$-$1Ca^{2+}(NH_2)$	922.45	221.4	624.5	816.01	557.5	802.4	820.92	367.8	788.1
$4Pb^{2+}$	923.27	221.4	624.5	817.38	557.5	802.4	822.28	367.8	788.1

The results show that for replacement the lead ion in places of sTnC needs more energy and the carboxyl conditions energy is more than amine. Dipole moments inter of calcium and lead in four places is the same by MM+ computational field approximately. The table 2 is the approaching steps of lead to different places of troponin C by seven steps that are calculated. In table 2, a lead ion approaches to the calcium ion in amine place of TnC from 8.78 °A distance which the total energy, dipole moment and RMS gradient are analyzed by MM+ field.

The total energy increases for approaching the lead ion to amine and carboxyl places to replace to calcium ion, shows the lead ion repelling from the around of troponin C. The dipole moment doesn't change by approaching lead ion, but RMS gradient is the most amounts in 2.05 °A distance. After that when a lead ion puts in amine place, approaching the second lead ion to another place of amine from 8.78 °A distance is simulated and its thermodynamic properties are calculated. In this step the most change is observed in 2.05 °A distance.

Table 2: Thermodynamics parameters of approaching of lead ion instead of calcium ions in NH_2-sTnC position.

Distance(Å)	$1Pb^{2+}(NH_2)$-$3Ca^{2+}$			$2Pb^{2+}(NH_2)$-$2Ca^{2+}$(COOH)		
	E_{total} mJ/mol	Dipole moment (D)	RMS kcal/mol·Å	E_{total} mJ/mol	Dipole moment (D)	RMS kcal/mol·Å
1.89	59.55	224.8	108.3	57.80	224.8	106.0
2.05	61.12	224.8	114.7	58.40	224.8	107.0
3.37	58.54	224.8	105.3	56.98	224.8	105.6
4.39	56.60	224.8	105.4	58.38	224.8	106.3
6.67	56.48	224.8	105.3	58.26	224.8	106.2
7.36	54.34	224.8	104.6	58.26	224.8	105.5
8.78	54.29	224.8	104.6	56.07	224.8	105.5
Distance(Å)	$3Pb^{2+}$-$1Ca^{2+}(NH_2)$			$4Pb^{2+}$		
1.89	56.89	224.8	105.5	56.02	224.8	105.1
2.05	57.50	224.8	106.4	56.63	224.8	106.1
3.37	56.08	224.8	105.0	55.21	224.8	104.7
4.39	57.48	224.8	105.7	56.25	224.8	105.4
6.67	57.35	224.8	105.7	56.29	224.8	105.3
7.36	55.21	224.8	105.0	54.02	224.8	104.6
8.78	55.61	224.8	105.0	54.14	224.8	104.6

In the third step, when two places of amine in troponin C are occupied by lead ions, movement of the third of lead ion in the first place of carboxyl for replacing with calcium ion is simulated and computed by seven distances. These reaction computational parameters are shown in table 2. In MM^+ filed the structural symmetry is very important. That is approximately changed by entering the third lead ion, as can be seen in their

computations. Noteworthy that in this step also the most changes are observed for the 2.05°A distance and the dipole moment of the structure doesn't change as well as RMS gradient has no considerable change.

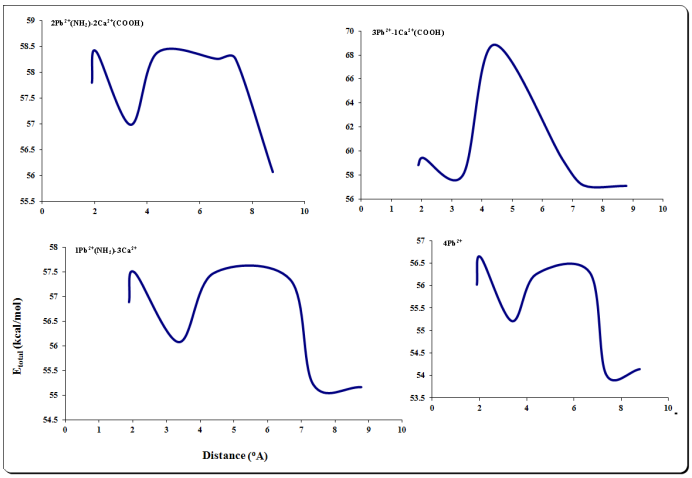

Figure 2: The total energy (kcal/mol) of approaching of lead ions instead of calcium ions in NH_2-sTnC position by different force files of calculation.

In the last step is the fourth lead ion approaches toward the calcium ion in the second place of carboxyl troponin C that this step is simulated and computed in 7 distances. The total energy is less than the previous step and the computed dipole moment is approximately the all steps. The most changes are observed in the 2.05 °A distance.

As could be seen in figure 2, lead ion approaches to troponin C in seven different distances and enters into amine and then carboxyl places respectively. It is because that electronegativity of amine places is more than carboxyl and the structural symmetry of the amine groups has sufficient spaces for lead ion entry. With the lead ion replacement instead of calcium ion, the energy increases, because is repellent troponin C to accept the new lead ion. This energy is necessary for re-absorbing lead ion that provides by glucose and increases lactic acid density around the muscles.

The coordination chemistry of proteins in interaction with toxic metals has been studied empirically, calculated results with the molecular mechanics method show good agreement to them [14].

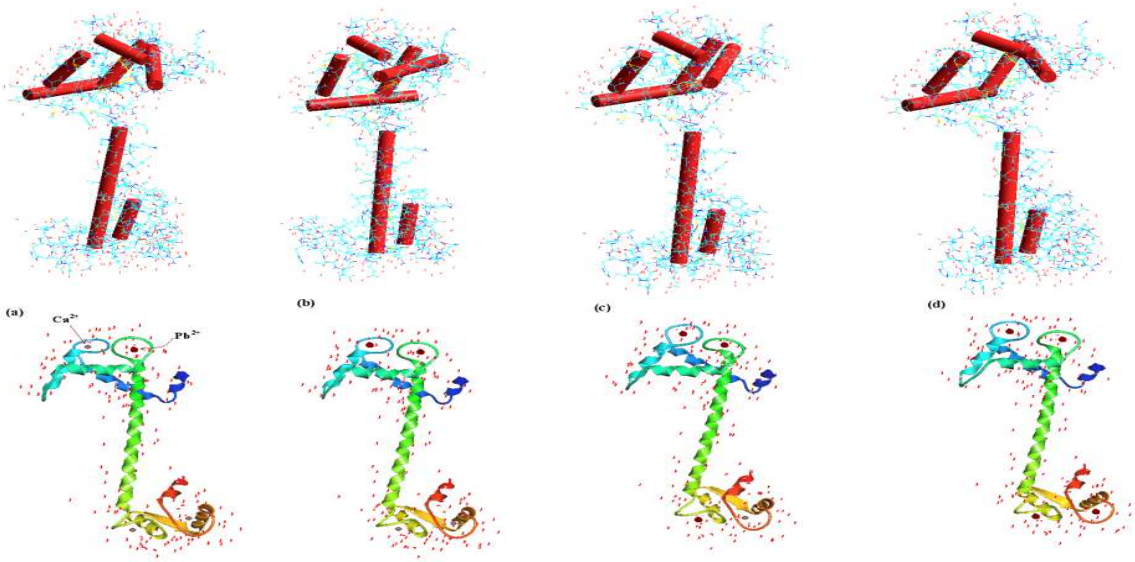

Figure 3: Alpha cylinders and view ribbon crystal structure of sTnC-Ions; a) one ion of lead in amine place and calcium ions are in other places ofsTnC, b) two ions of lead in amine places and calcium ions are in other places of sTnC, c) three lead ions in amine and carboxyl places and d) sTnC with four Pb^{2+} in N and C-terminal, approached of lead ions to institute Ca^{2+} ions in sTnC then can be seen rotation in it.

It can be seen in figure 2 that the total energy has a sudden increase in 2.05 °A distance and a significant decrease in 3.37°A distance because these fields are used for biological and ecological structures. MMx fields are generally used to structure and thermodynamic studies such as surfaces of potential energy, links and angles in small polar molecules. By entering a new lead ion in polar structure of sTnC, some changes are observed, as are shown in figure 3.Figure 3 shows the alpha cylinder and ribbon models of troponin C. At first, a lead ion is replaced to calcium ion, and then all places are occupied by ions of lead. When lead ions are replaced, the whole structure of troponin C rotates in clockwise. So TnC for leaving of lead ions from amine and carboxyl places should be to consume more energy.

Entering 210 metal to sites of protein metal binding is investigating an experimental study that change direction and angles between amino acids is confirmation of the results of this study [15]. With entry of lead ions in the places of TnC, structural changes of TnCis greater and stronger than interaction with calcium ions, which is causing muscle contraction and pain [16].

- **Effect of lactic acid concentration**

To provide energy to muscles, glucose is consumed in presence of oxygen to and converts to adenosine triphosphate (ATP), water and CO_2. In the rapid movements of muscles such as walking or other activities, there is not enough oxygen for oxidative metabolism of glucose leading to glucose converted to pyruvic acid and then to lactic acid.

During recovery, the lactic acid by NAD (Nicotinamide adenine dinucleotide (NAD) is a coenzyme and converts to pyruvic acid and is stored in glycogen form in muscle or liver as an energy source [17]. It can break down to tow pyruvic acid molecules and provides four molecules of ATP. The muscle fatigue results from creatine phosphate (CP) depletion and, specifically, the increases of inorganic phosphate and ADP that occur when the muscle supply of CP is reduced [18].

In this study, we computed the thermodynamic parameters of increasing concentration of the lactic acid molecules around troponin C during the contraction and the results are presented in table 3. When the Pb^{+2} ions tear to NH_2 and COOH, contraction is occurring. In other words it decreases the angles between sTnC atoms. This change in configuration provides the necessary energy for link TnI-TnC formation to turn the actin of skeletal muscle by Tm-TnT.

Table 3: Thermodynamics parameters of increasing the number of lactic acid molecules around in replacementof leadionsinsteadof calcium ions in N & C terminals-sTnC positions.

%Wt Lactic acid	MM^+			Amber			BIO^+(Charmm)		
	E_{total} (mJ/mol)	Dipole moment (D)	RMS kcal/mol·Å	E_{total}(mJ/mol)	Dipole moment (D)	RMS kcal/mol·Å	E_{total}(mJ/mol)	Dipole moment (D)	RMS kcal/mol·Å
$1Pb^{2+}(NH_2)$-3Ca									
5	131.78	230.9	1107	153.85	562.8	1571	314.05	350.3	1557
10	132.12	236.7	1097	154.49	565.5	1557	314.41	363.00	1543
15	132.44	242.7	1087	155.12	571.4	1543	314.76	373.00	1529
20	175.84	248.9	1079	259.74	606.6	3557	317.02	392.70	1718
$2Pb^{2+}(NH_2)$-$2Ca^{2+}$(COOH)									
5	1219.60	231.2	786.7	142.26	564.60	1547.00	187.69	369.3	1523.00
10	75.29×10^4	236.9	780.9	124.36×10^4	572.40	6.26×10^3	210.58	375.2	3.73×10^3
15	84.64×10^4	242.9	954.0	1.86×10^6	557.30	2.77×10^3	274.51	365.7	1.11×10^3
20	84.65×10^4	249.1	947.0	1.86×10^6	556.80	2.75×10^3	281.40	353.9	1.10×10^3
$3Pb^{2+}$-$1Ca^{2+}$(COOH)									
5	37.87×10^4	231.2	788.4	29.55×10^4	560.5	9.81×10^4	231.97	379.4	1.37×10^4
10	38.44×10^4	236.9	782.0	30.53×10^4	593.0	9.73×10^4	241.81	395.2	1.36×10^4
15	14.79×10^5	242.9	785.6	72.86×10^4	576.3	8.34×10^4	295.19	382.7	1.70×10^4
20	14.85×10^5	249.1	780.5	72.34×10^4	571.5	8.31×10^4	325.19	376.2	1.69×10^4
$4Pb^{2+}$									
5	70.30×10^4	231.2	7912	36.50×10^4	566.2	3.11×10^4	217.97	368.5	6.56×10^4
10	74.24×10^4	236.9	787.9	37.86×10^4	558.2	3.08×10^4	288.00	364.3	6.51×10^4
15	26.32×10^5	242.9	495.3	4.08×10^5	549.5	1.46×10^5	411.32	359.6	9.42×10^4
20	26.35×10^5	249.1	4912	4.08×10^5	555.5	1.45×10^5	460.79	368.6	9.34×10^4

With increase of molecule number of lactic acid around troponin C, the total energy (mJ/mol), dipole moment (D) increased, but the computed potential energy for this concentration change around sTnC. At first increased and when acid lactic gets to 20 weight percent, the total energy increase significantly.

As can be seen in table 3, in these reactions total energy increases with increasing lactic acid concentration. The dipole moments are increasing that indicts the configuration change and transferring all atoms of TnC. Also RMS gradient changes with pH, but a significant change is observed in it when the acid, lactic molecule number reach to 20 weight percent. The concentration of H ion increases with increase of lactic acid. Under this

condition muscle and blood acidity increase and are disturbed enzyme reactions [19]. It causes prolonged spasm, that is a consequence of fatigue as well as release of Pb^{+2} on sTnC would be delayed.

With increase of the lactic acid molecule number around troponin C, the total energy for a lead ion in amine place is constant initially (table 3) and then increase, but the total energy for replacement of second and third lead ions, decreases at first, then increase significantly, by increasing the lead ions in NH_2 and COOH places, the dipole moment decreases, so the sTnC structure will be symmetrical. Increasing the total energy affirms it. The dipole moment (D) increases, because of increasing the lactic acid around troponin C causes molecular polarity increases too. The total energy in the computational field of Amber and BIO+ is much more than MM+ field. During the physical activity, for a few minutes, lactic acid level gets to 180 ml percent and pH in blood and muscle close to 7 and 6.4 respectively. it is the increased acidity in tissues, due to the buildup of hydrogen ions, that contributes to the sensation of fatigue [16]. Alternative work lactic acid importance is that 2 moles of ATP are recycled quickly and are access able for hardworking muscle to do heavy and fast activities, that causes the easily replacing of lead ion instead of calcium[20].

Table 4: Thermodynamics parameters of replacement of lead ions instead of calcium ions in NH_2& COOH-sTnC positions at different temperatures.

T (K)	MM^+			Amber			BIO^+(Charmm)		
	E_{total} mJ/mol	Dipole moment (D)	RMS kcal/mol·Å	E_{total} mJ/mol	Dipole moment (D)	RMS kcal/mol·Å	E_{total}mJ/mol	Dipole moment (D)	RMS kcal/mol·Å
					$1Pb^{2+}(NH_2)$-3Ca				
298	241.47	234.30	174.70	197.16	230.1	134.30	223.48	230.2	144.50
300	241.54	230.00	141.70	197.24	230.1	134.30	227.46	230.2	144.50
310	241.92	228.80	176.10	197.61	230.1	134.30	229.93	230.1	144.50
320	242.30	228.80	176.10	197.99	230.1	134.30	302.30	230.1	144.50
					$2Pb^{2+}$(NH_2)-$2Ca^{2+}$(COOH)				
298	251.48	235.00	151.00	220.00	235.7	138.5	280.48	235.8	144.4
300	251.56	235.40	151.90	220.32	235.7	138.4	297.63	235.8	144.4
310	251.94	234.30	174.60	220.71	235.7	138.4	322.50	235.8	144.5
320	252.33	234.30	174.60	221.09	235.7	138.4	322.50	235.8	144.5
					$3Pb^{2+}$-$1Ca^{2+}$(COOH)				
298	304.47	240.10	173.30	284.06	241.4	147.3	320.04	241.6	154.5
300	304.57	241.20	156.90	284.21	241.4	147.3	322.50	241.6	154.5
310	304.97	240.10	173.10	284.41	241.4	147.3	323.07	241.6	154.5
320	305.36	240.00	173.10	284.81	241.4	147.3	323.46	241.6	154.5
					$4Pb^{2+}$				
298	334.68	246.10	171.80	291.15	247.1	146.1	321.55	247.7	158.6
300	334.76	247.10	164.70	291.25	247.1	146.1	322.06	247.7	158.6
310	335.16	264.10	171.60	291.50	247.1	146.1	325.59	247.7	158.6
320	335.56	246.10	171.60	291.90	247.1	146.1	325.98	247.7	158.6

- **Results in temperature rise**

The human body is active in a limited core temperature of approximately 37 to 39 ° C. Normally, the body is increasing the repelling of temperature, can balance the internal environment. When the temperature of body is high, the water of the body decreases by sweating, so the body's ability to control its temperature decreases, the body may be heat stroke. Now we are studying the effects of increased temperature on replacing lead ions to calcium ions. To better understand the effect of increased temperature, the thermodynamic parameters of sTnC are studied and computed at temperature of 298 to 320K, the results show in table 4. The central temperature of the human body is 37 to 39 ^oC that by decreasing 1 degree from 37 ^oC , the total energy and other computational parameters for this reaction increase, that causes trembling in order to increase the body temperature, and also by decreasing the temperature, contraction rate and muscle power decrease significantly.

Now by gradually increasing the temperature on sTnC during contraction from 37 ^oC the total energy and other thermodynamic parameters increase. Increase of the body temperature causes to increase in oxygen and glycogen consumption, and increased level of lactic acid in muscles lead to fatigue. In MM+ field, increase in temperature caused to insignificant increase of dipole moment also RMS gradient increase.

Conclusion

Troponin C including amino acids that are considered as a macromolecule, and according to the high volume of molecule, computing is so time–consuming. The troponin structure and interaction with lead ions through DFT method on the basis B3LYP/6-31G are optimized. The thermodynamic parameters show that this interaction is endothermic, that entering the lead ion, turning of sTnC is much more than entering calcium ion. So the lead ions reabsorbing is harder and need much more energy, that are the cause of pain in muscles, as one of the symptoms of entering ion into the body, like respiration, using cosmetics and polluted grains.

By entering the lead ion, upper part of sTnC is computed and the lower part of this macromolecule is opened that leads to other troponin turning and provides. The actin head and myosin link more energy are needed because of more truing of the molecule to reabsorb the ion and to return to the initial case, this causes to increase the lactic acid density and the temperature around the muscles. This is caused high fatigue the person who is exposed to lead ions.

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