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Study of pH influence on the stability of 175th codon of *P53* genes by computational and modeling methods

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ABSTRACT

P53 tumor suppressor gene, also known as "genome guardian" is mutated in more than half of all kind of cancers. In this study we have investigated the controls of environmental pH for P53 gene mutation in point of specific sequence which is prone to mutagenesis. The most probable cancerous mutations occur as point mutations in exons 5-8 of P53 gene. The 175th codon of P53 is the third most mutated codon in this gene. By experimental research, it is revealed that acidic pH raised the rate of cancer and mutation in 175 CGC codon of P53 gene. It to some extent is due to protonation of this three nucleotide codon. Mutation in this codon changes the encoding amino acid and subsequently produces a protein which has oncogenic features instead of tumor suppressor characteristics of original p53 protein. In current study, we perform investigation on the impact of protonation on stability of codon 175CGC in this gene. We used HyperChem software for answering to our mentioned goal above. Our results suggested a reliable answer about the effect of protonation on mentioned codon and its stability. From theoretical point of view, acidity can decrease the instability of this specific codon. Along with the experimental investigations, our results can to some extent elaborate acidic pH competency to cause mutation.

Keywords: P53 gene; Protonation; Mutagenesis

INTRODUCTION

P53 is a tumor-suppressor protein which has classical features of transcription factor. It responses to different cellular stress via inducing activation or repression of more than 2500 genes [1]. Because of its critical role in protection against cancer, it is called "the guardian of genome". Somatic mutation of this gene has been observed in more than 50 percent of different human cancers [2]. Unlike other tumor suppressor genes (like *RB*, *APC* and *BRCA1*) in which inactivation occurs by frame shift and nonsense mutations, about 80 percent of *P53* mutations are missense [3]. In *P53* gene the most prevalent site of mutagenesis is along 5-8 exons. In these exons the most mutable nucleotides are deoxycytidine and deoxyguanosine (CpG) dinucleotide which are the most important mutational target in human cancers [4].
The 175 codon of *P53* gene is the third most mutable codons and this mutation frequency is 6.1 percent of all mutations observed in *P53* gene [4].

The importance of studying mutational pattern is to better understand the function

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of p53 domains, their effects on tumor-suppressing properties of p53 and the nature of etiologic substances as environmental etiologic biomarkers [5].
Furthermore, medical studies about the reasons of carsinogenesis suggest that the acidic pH can be considered as a condition causing cancers. For instance, a report showed that the pH of tumor microenvironment is more acidic than normal cells [6]. It is shown that disease "Barrett's esophagus" is related to acid reflux [7] and even in its early stage mutation in *P53* has been observed [8].
Although the role of acidic pH is significant in carcinogenesis, its molecular mechanism is little known. It is known that physical properties of DNA is crucial for molecular genetic studies [9]. DNA molecule is constantly exposed to a great range of physical and chemical substances which harm its structure [10].

Over 30 years, a great number of researches were done by using biological experimental methods on *P53*. Hence conducting research theoretically and practically on *P53* and the conditions causing mutation in it can help us to be successful in prevention and treatment of cancer [11, 12].

The effect of pH on DNA structure is not fully evaluated, because of the difficulty of measuring this quantity in cellular nucleus. In this regard, simulation and computational chemistry could be helpful. In current study, we tried to evaluate a new carcinogenesis pathway caused by pH alteration and causes missense mutation. We specifically focused on 175 codon of *P53* gene (Figure 1).

THEORETICAL METHOD

CGC three-nucleotide was drawn as a double stranded DNA structure by HyperChem™. Then the structure was optimized by geometric optimization order. To determine the effect of pH on this structure a periodic box with 30 °A dimensions was designed. In addition, according to pH corresponding H+ and OH- for pH values 6.8 and 7.4 were respectively located within a periodic box. Simulation was done in MM+, AMBER, BIO+ and OPLS force fields. Molecular Mechanics calculations were assessed by Monte Carlo method [13]. Three important energy parameters – kinetic energy, potential energy and total energy- in four different simulating temperatures (308, 310, 312 and 314 Kelvin) were used for computation.

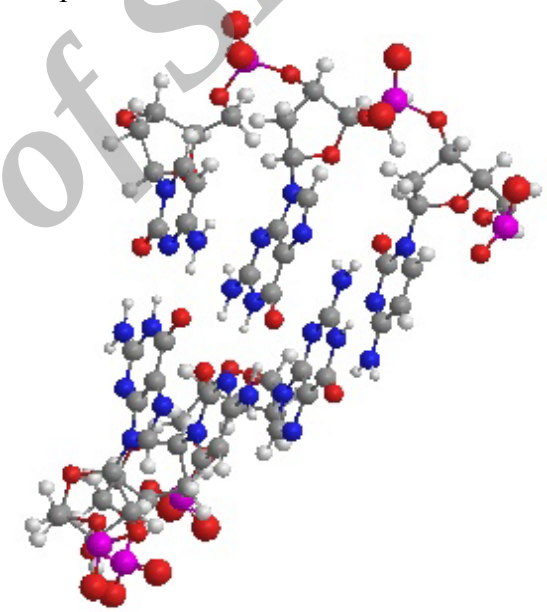

Fig. 1. Molecular structure of CGC three-nucleotide.

RESULTS AND DISCUSSION

More than half of all human cancers have mutation in *P53* gene. Theoretically evaluation of environmental factors like pH can help us to understand the causes of mutation and its molecular mechanisms. In current study computations were done in sophisticated and appropriate molecular modeling environment of HyperChem™ which is well known for its quality and

flexibility [14, 15].

It is known that atoms are held together by forces. Function of biological systems arises from interaction of resilient bonds between atoms and electron motion. The main purpose is to seek for the lowest energy, in which the molecule is in its most stable state [16, 17]. In this study AMBER, MM^+, BIO^+ and OPLS force fields were chosen. When CGC is modeled, it undergoes shaking, rotating, stretching, and etc. functions around its bonds. The total potential energy is the sum of mentioned contribution interactions based on the force fields.

MM^+ is a proper parameter for attaining vibration motion of atoms, related bond stretching potential, and angles bending. AMBER force field has extensive application for proteins and nucleic acids. It assigns all conformational energies and treats with hydrogen bond energy, and torsion term [18]. Like AMBER, OPLS is designed for computation of proteins and nucleic acids. In this force field bonded potentials are similar to AMBER and its non-bonded potentials involves vander waals and electrostatics. BIO^+ filed is an extended form of CHARMM. Similar to AMBER and OPLS it has been designed to study macromolecules [19].

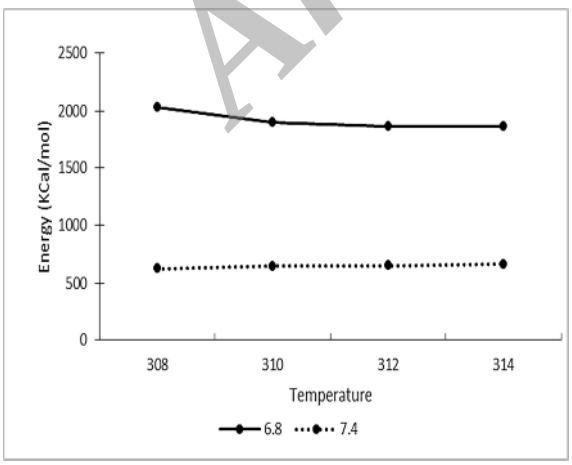

Fig. 2. Comparison of minimum energy level in different temperatures in BIO^+ force field.

CGC codon was simulated in mentioned force fields in 4 different temperature (308K, 310K, 312K and 314K).

According to results observed in table 1, it is clear that potential energy of acidic pH is higher than physiologic pH and this increase can be observed in different force fields. In figure 2 minimum potential energy calculated by BIO^+ force filed in pH 7.4 and 6.8 have been reported. The increase of potential energy can be observed in this graph. It is notable that molecular stability in physiologic pH is more. Minimum potential energy levels in normal body temperature were 1898.27 Kcal/mol and 645.46 Kcal/mol for pH 6.8 and 7.4, respectively which shows that in acidic pH energy level increased about 3 times.

Data obtained for kinetic energy have been presented in table 2. As the temperature was been raised, the amount of kinetic energy constantly increase. Obtained figures for kinetic energy in various time steps and different force fields were constant and the maximum quantity observed in 314 K, 212.46 and 241.48 Kcal/mol for pH values of 7.4 and 6.8, respectively. It is known that to have optimum function in biologic system, the energy levels must be in the minimum level.

Data analysis of table 3 exhibited that total energy quantities were affected by decrease of pH value and increase of temperature.

CONCLUSION

Current study was done to evaluate the thermodynamic role of acidic pH in developing mutation in 175th codon of *P53* gene. Increase of energy level has been observed in potential, kinetic and total energies. Energy increase leads to molecular instability. From data obtained in current study, it can be concluded that acidic condition in cellular environment

directly produce unstable codon structure which can alter the structure of the codon. If this change is not repaired by biologic system, it can end to the stabilized alteration (also known as mutation) in DNA. Because of the fact that mutations usually have negative effect on proliferation of the cells, they can lead to cancer progression. If these mutations occur in genes like *P53*, the meaning of that is unlikely to be treated cancer.

Table 1. Computed CGC potential energy (kcal/ mol), belong to AMBER, MM^{+}, BIO^{+} and OPLS force fields under four different temperature and 2 various pH values

	Potential Energy (Kcal/mol)															
Method	Amber/Monte Carlo				Amber/Monte Carlo				MM^{+}/ Monte Carlo				MM^{+}/ Monte Carlo			
pH condition	6.8				7.4				6.8				7.4			
time (PS)	308 K	310 K	312 K	314 K	308 K	310 K	312 K	314 K	308 K	310 K	312 K	314 K	308 K	310 K	312 K	314 K
10	30946.9	35880.4	36071.1	34180.7	657.9	675.7	644.8	664.7	11138.3	11204.4	11230.5	11286.3	958.0	959.1	959.1	954.9
20	8683.7	8154.7	8961.0	9654.5	589.0	591.7	562.3	583.0	7025.4	6719.9	7012.7	7150.2	672.2	667.4	648.4	649.4
30	5556.9	5334.8	5653.5	5746.6	543.6	551.2	526.9	546.0	4816.6	4711.1	4892.5	4887.9	595.6	584.6	568.2	570.4
40	4331.4	4120.6	4335.6	4414.7	509.6	511.2	490.0	524.2	3698.2	3639.8	3733.9	3774.5	538.6	546.2	531.0	534.7
50	3569.1	3370.9	3530.4	3554.0	481.9	504.0	477.0	486.8	3006.6	3031.6	3020.2	3068.9	505.7	511.2	508.7	502.8
60	3050.4	2898.7	2954.3	2923.6	461.2	482.5	445.9	482.3	2561.4	2557.3	2583.7	2581.6	490.9	490.8	494.7	497.4
70	2636.0	2521.4	2520.7	2536.8	450.5	473.0	446.2	478.8	2220.1	2191.1	2275.0	2212.5	475.9	479.7	489.9	482.3
80	2356.5	2244.9	2255.8	2269.0	443.4	457.3	442.1	467.0	1970.1	1935.2	2013.4	1974.9	465.8	454.8	476.7	480.9
90	2141.5	2027.8	2052.5	2044.5	432.8	449.2	430.5	456.3	1755.9	1718.5	1817.2	1767.0	450.2	455.5	472.7	461.3
100	1947.4	1867.5	1877.5	1859.9	417.2	441.8	439.3	460.5	1591.5	1564.9	1630.7	1595.5	443.4	450.6	465.0	467.5
Method	BIO/Monte Carlo				BIO/Monte Carlo				OPLS/ Monte Carlo				OPLS/ Monte Carlo			
pH condition	6.8				7.4				6.8				7.4			
time (PS)	308 K	310 K	312 K	314 K	308 K	310 K	312 K	314 K	308 K	310 K	312 K	314 K	308 K	310 K	312 K	314 K
10	76736.2	49314.0	55623.5	46280.4	952.8	954.3	937.6	954.7	40460.0	39921.5	40241.2	39096.5	1419.3	1404.8	1416.5	1401.1
20	12149.5	11623.8	13179.2	9677.8	809.2	828.1	829.8	823.5	35048.2	35042.5	34891.1	34765.8	1277.5	1276.5	1280.7	1276.9
30	6973.6	6406.8	6718.9	6054.1	736.2	762.1	778.5	767.9	33834.7	33992.0	33927.1	33900.5	1224.6	1238.2	1236.5	1232.3
40	5063.6	4692.4	4690.6	4614.3	706.4	720.8	732.6	737.5	33363.2	33388.3	33454.3	33452.6	1192.0	1203.2	1196.8	1208.9
50	4188.2	3736.0	3741.2	3671.3	682.6	681.3	710.9	706.1	33022.9	32982.2	33086.9	33128.8	1176.1	1170.6	1173.5	1172.4
60	3573.8	3098.0	3102.0	3071.1	669.3	664.0	692.4	698.0	32708.8	32689.0	32767.6	32825.5	1144.1	1161.1	1154.4	1152.1
70	3023.0	2706.8	2629.2	2662.1	674.7	657.9	681.6	671.1	32415.2	32375.1	32486.3	32556.3	1130.4	1133.8	1136.4	1139.0
80	2654.1	2411.0	2314.4	2355.4	650.5	639.5	672.3	670.5	32138.5	32103.4	32224.2	32304.9	1122.7	1123.6	1125.1	1117.4
90	2360.4	2175.1	2089.7	2108.5	637.6	626.6	660.4	625.6	31839.1	31845.3	31980.5	32046.1	1102.6	1110.8	1104.7	1101.9
100	2127.2	1972.5	1934.3	1917.7	627.2	635.1	650.5	661.7	31563.0	31604.7	31748.1	31779.2	1093.8	1105.8	1088.4	1105.9

Table 2. Computed CGC Kinetic energy (kcal/ mol), belong to AMBER, MM^{+}, BIO^{+} and OPLS force fields under four different temperature and 2 various pH values

	Kinetic Energy (K Cal/mol)							
pH condition	6.8				7.4			
Method	308 K	310 K	312 K	314 K	308 K	310 K	312 K	314 K
AMBER	209.3228	210.6821	212.0413	213.4005	174.4357	175.5684	176.7011	177.8338
BIO $^{+}$	209.3228	210.6821	212.0413	213.4005	174.4357	175.5684	176.7011	177.8338
MM $^{+}$	209.3228	210.6821	212.0413	213.4005	174.4357	175.5684	176.7011	177.8338
OPLS	209.3228	210.6821	212.0413	213.4005	174.4357	175.5684	176.7011	177.8338

Table 3. Computed CGC total energy (kcal/ mol), belong to AMBER, MM^{+}, BIO^{+} and OPLS force fields under four different temperature and 2 various pH values

Total Energy (Kcal/mol)																
Method	Amber/Monte Carlo				Amber/Monte Carlo				MM^{+}/ Monte Carlo				MM^{+}/ Monte Carlo			
pH condition	6.8				7.4				6.8				7.4			
time (PS)	308 K	310 K	312 K	314 K	308 K	310 K	312 K	314 K	308 K	310 K	312 K	314 K	308 K	310 K	312 K	314 K
10	31155.3	36090.2	36282.2	34393.2	831.4	850.33	820.6	841.62	11347	11414.2	11441.6	11498.7	1131.6	1133.7	1135.2	1131.8
20	8892.092	8364.46	9172.12	9866.92	762.53	766.38	738.1	759.85	7233.8	6929.63	7223.82	7362.66	845.67	842.09	824.22	826.255
30	5765.305	5544.57	5864.66	5959.02	717.13	725.88	702.7	722.85	5025	4920.87	5103.62	5100.39	769.15	759.26	743.99	747.323
40	4539.845	4330.32	4546.69	4627.13	683.1	685.8	665.7	701.1	3906.6	3849.54	3945.01	3986.96	712.16	720.84	706.82	711.636
50	3777.551	3580.67	3741.52	3766.46	655.37	678.66	652.8	663.72	3215	3241.38	3231.27	3281.38	679.18	685.83	684.44	679.66
60	3258.767	3108.43	3165.37	3136.11	634.75	657.12	621.7	659.15	2769.8	2767.02	2794.83	2794.03	664.45	665.48	670.47	674.311
70	2844.415	2731.16	2731.82	2749.23	624.04	647.64	622	655.74	2428.6	2400.86	2486.13	2424.97	649.44	654.37	665.63	659.201
80	2564.953	2454.66	2466.86	2481.49	616.89	631.95	617.9	643.89	2178.5	2145.01	2224.53	2187.35	639.28	629.42	652.45	657.762
90	2349.892	2237.52	2263.61	2257	606.33	623.82	606.3	633.21	1964.3	1928.22	2028.3	1979.49	623.77	630.18	648.46	649.427
100	2155.785	2077.22	2088.56	2072.35	590.77	616.48	615	637.39	1799.9	1774.67	1841.76	1807.98	616.95	625.25	640.74	644.388
Method	BIO/Monte Carlo				BIO/Monte Carlo				OPLS/ Monte Carlo				OPLS/ Monte Carlo			
pH condition	6.8				7.4				6.8				7.4			
time (PS)	308 K	310 K	312 K	314 K	308 K	310 K	312 K	314 K	308 K	310 K	312 K	314 K	308 K	310 K	312 K	314 K
10	76944.61	49523.7	55834.7	46492.9	1126.3	1128.9	1113	1131.6	40697	40159.9	40481.1	39337.2	1592.8	1579.5	1592.3	1578.03
20	12357.94	11833.5	13390.3	9890.29	982.74	1002.7	1006	1000.4	35285	35280.9	35131	35006.5	1451	1451.2	1456.5	1453.75
30	7181.998	6616.51	6930.01	6266.54	909.72	936.71	954.3	944.85	34072	34230.4	34167	34141.3	1398.1	1412.8	1412.3	1409.16
40	5272.004	4902.11	4901.71	4826.73	879.91	895.45	908.4	914.41	33600	33626.7	33694.2	33693.3	1365.5	1377.8	1372.6	1385.76
50	4396.604	3945.8	3952.33	3883.8	856.13	855.94	886.6	882.97	33260	33220.6	33326.8	33369.5	1349.6	1345.2	1349.2	1349.3
60	3782.251	3307.72	3313.09	3283.54	842.77	838.67	868.2	874.89	32946	32927.4	33007.5	33066.2	1317.6	1335.8	1330.2	1329.05
70	3231.405	2916.61	2840.26	2874.55	848.23	832.59	857.4	848.01	32652	32613.5	32726.2	32797	1304	1308.4	1312.1	1315.89
80	2862.476	2620.72	2525.49	2567.85	823.98	814.17	848.6	847.4	32375	32341.8	32464.2	32545.6	1296.2	1298.2	1300.9	1294.31
90	2568.836	2384.82	2300.81	2320.97	811.12	801.27	836.2	829.52	32076	32083.7	32220.4	32286.8	1276.1	1285.5	1280.4	1278.82
100	2335.641	2182.29	2145.39	2130.18	800.68	809.79	826.2	838.64	31800	31843.1	31988	32019.9	1267.3	1280.4	1264.1	1282.76

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