International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 10, Issue 7, 2018

Original Article

MOLECULAR DYNAMICS STUDY OF NON-HYDROGEN-BONDING BASE-PAIR DNA DUPLEX d(GTCD$_{NAM}$ GCGCCGTGGC). d(GCCACGGCGCD$_{5SICS}$GAC)

RAKESH KUMAR TIWARI

DDU Gorakhpur University, Gorakhpur
Email: drrkt@yahoo.com

Received: 18 Jan 2018 Revised and Accepted: 15 Jun 2018

ABSTRACT

Objective: The objective of the study was to elucidate the structural activity of a natural DNA sequence modified by a hydrophobic base-pair which didn't form Watson-Crick (W-C) hydrogen-bond. The modified unnatural base-pair (DNAM-D5SICS) was introduced in DNA sequences of 14-mer for molecular dynamics study in water solution.

Methods: A 200 ns molecular-dynamics-simulation in orthogonal-box-water-solvent, using Particle-mesh-Ewald-method (PME) within periodic-boundary-condition (PBC) was performed by using AMBER-14 code. The force-field-ff12SB-force-field was used during the simulation while the force-field-parameters of modified base-pair, compatible to ff12SB-force-field, were calculated by Gaussian-09-code using *ab-inito*/Hartree-Fock-methodology. The code CPPTRAJ, (a module of AMBER-14) CURVE and Chimera were used in the analysis of the data.

Results: Root mean square deviation (RMSD) of heavy atoms of the trajectory revealed that the structure of the equilibrated duplex was stable, sequence-dependent and had mixed DNA-conformation. A little distortion near to the neighbor of the modified base-pair in the duplex strand was observed. However, we got a stabilized structure of such type of duplex if we placed modified base-pair after the third place in the strand.

Conclusion: The study concluded that the distortion produced by modified-base-pair in the overall structure of duplex was local while the confirmation of such type of duplex was mixed and maintained the Watson-Crick (W-C) integrity of DNA. The study would help in the use of hydrophobic base-pair materials in biotechnological applications and the understanding of their structure-function relationship.

Keywords: H-bond, Molecular Dynamics, Modified Hydrophobic Nucleotide (MHN), Unusual DNA, Modified DNA, Non-hydrogen-bonding-base-pair, and Unnatural Base-pair (UBP)

DOI: http://dx.doi.org/10.22159/ijpps.2018v10i7.24841

INTRODUCTION

In recent years the use of modified hydrophobic base-pair has been developed as the most fascinating technique in the genetic engineering [1, 2]. The basic principle involved in the selection of the base-pairs is that "a crucial position in the base-pair has to be hydrophobic for the enzymes in DNA-function, yet it also has to accept hydrogen-bonds if enzymes are replicating the strand" [3–5]. However, the set principle is that water participates in all biological processes involving folding, recognition or catalysis of nucleic acids". Hydrogen-bonding properties of water which allow liquid-water to execute an intricate three-dimensional synchronized ballet exchanging partners while retaining complex-order and enduring-effects is the root cause of this versatility [6].

Fig. 1: D$_{SICS}$-M$_{MO2}$ and D5$_{SICS}$-D$_{NAM}$ base-pair

Romesberg *et al.*[7, 8] has successfully engineered replications of some sequences of nucleic acid using hydrophobic base pairs, and they found 60 base-pairs out of about 3,600 combinations which prove the earlier principle to some extent. The base-pair D$_{MMO2}$-D$_{SICS}$ was found more appropriate for pairing as it shows two contradictory characteristics, hydrophobic for enzymes into DNA-function and accepts hydrogen-bond when enzymes are replicating the strand. In the refining process of these base-pairs the base-pair D$_{NAM}$-D5$_{SICS}$ (fig. 1) was found to be most efficient which was synthesized by modifying base-pair D$_{MMO2}$-D$_{SICS}$[8] Its hydrophobic characteristics maintained helix integrity and hydrophilic the central dogma function; just like switching device at the key position of the mechanism of the central-dogma [7] The profound insight, received about modified base-pair D$_{NAM}$-D5$_{SICS}$, has been utilized in the study of some crystal structures of KlenTaq-DNA-polymerase which was the large fragment of the type I DNA-polymerase from Thermus-Aquaticus when made the complex with a templating D$_{NAM}$ with or without bound D$_{5SICS}$TP [9]. Similarly, in a NMR study on 12-mer 5'-CCTTTCD5SICSTTCTC-3' reveals solution-conformation of the possibility [9]. The structural conformational details of the study have reached on following conclusions [8, 10] (i) The mechanism of the insertion of DNAMTP opposite d5SICS was less efficient than the insertion of D5$_{SICS}$TP opposite D$_{NAM}$ in starting replication.(ii) The nucleobases paired in an intercalated manner, similar to their pairing in free duplex DNA and the mode of pairing depends on the length of the single-stranded template, and (iii) more importantly, the mode of intercalation appeared to depend mostly on sequence-specific interactions of the flanking nucleotides, with the specific packing interactions between the intercalating nucleobases of secondary importance. These conclusions are drawn on the basis of the few crystallographic and experimental results [10–13]. And are limited to an insertion of D$_{NAM}$ and 5$_{SICS}$ in XACCAGGGCGCY (12-mer primer, and corresponding templet and 'X' represents purines and 'Y' represents DNAM or D5SICS) in different crystal and NMR

experiments. In view of the biotechnological importance of such type of material necessitates the generalization of such type of expanded genetic code which needs more physical parameters. Molecular dynamics simulations is one of the most emerging methodologies in structural and molecular biology, and widely used to explore the structure, conformational aspects and binding affinities of biomolecules [14–18]. As an attempts to characterize the conformation of hydrophobic, unnatural base-pair, the conformational parameters of the complexes leading to the insertion of D_{NAM} opposite to D_{5sics} in DNA-duplex we modelled 14-mer DNA strands of sequences d(GTCD_{NAM} GCGCCGTGGC). d(GCCAC-GGCGCD_{5SICS}GAC) in intrinsic solution and is reported here.

MATERIALS AND METHODS

Initial structures of molecules were modelled by NAB and CHIMERA-code [19–21] assuming B-DNA conformation of the sequence by using the parameters from the Arnott fibre diffraction model [19]. The simulation was performed in the presence of 10 Å thickness of solvent (TIP3P water molecules) around duplex in an orthogonal box of density 0.739 g/cc at atmospheric pressure and room temperature. Orthogonal-box was set to be the unit in periodic boundary condition for further calculation. F19SB-force-field, a non-bonded-cut-off of 10 Å in direct space, were employed to treat long-range-electrostatics interaction by Particle-Mesh-Ewald method (PME)[[22, 23]. In order to keep the parameters consistent with amber-force-field, the force-field parameters for MD simulation of D_{NAM}-$D5_{SICS}$ base-pair was calculated by using ab-initio Hartree-Fock/QM methodology with 6-31G* basis set [24, 25] In which atom–centred-point-charges of the deoxyribose-nucleotides were calculated by using recommended multiple-molecular-fitting and restrained-electrostatic-potential-fitting (RESP) [26]

Simulation process involved the following steps: a)-Minimisation b)-Restrained-dynamics to Heat the system from 0-K to 350-K and then 350-K to 300-K, c)-Restrained-dynamics of 10 ns time interval in constant-volume in which restrained-force was gradually decreased by 100 kcal/mole to 0 kcal/mole in 6 steps of 20 kcal/mole/step, d)-Constant-pressure-dynamics of 10 ns to equilibrate the system at 300 K, 10 ns dynamics to fully equilibrate the system and e)-200 ns production-dynamics. During initial steps (up to equilibrate the system) of the dynamics, Shake-algorithm of 1.0 fs time-step was used to constrain Hydrogen-atoms however, a 2.0 fs time-step was used for production-dynamics. Langevin-coupling with collision-frequency 2.0 was being used for temperature-regulation during dynamics. The computed data was analysed by using the CPPTRAJ module of AMBER-14-code. Standard angles and helicoidal parameters were determined by using algorithm adapted by Babcok *et al.* and reference-frame-coordinate was used from Olsen *et al.* [27–29]. Similarly, the helical parameters of modified-base-pairs were defined by the set of atoms of modified base-pair corresponding to the natural base-pair A-T. Nucleic acid residue names are referred to in the text as one-letter codes with a residue number; residue numbers in the 5′ to 3′ directions are represented by 1-14 for the first strand and, 15-28 for the second strand of the duplex (14-mer sequence) respectively. Next-29 numbers represent the residue number of ions in the solution for duplex respective. All the molecular graphics images were produced using CHIMERA package [30]. Several macros have been developed to make the AMBER data compatible with the CHIMERA module.

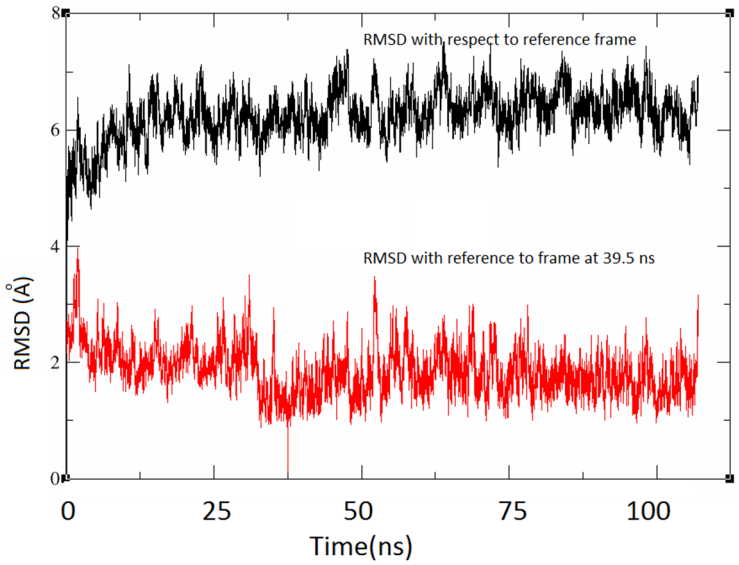

Fig. 2: RMSD with reference to crystal structure (Black Color) and with reference to 39.5 ns structure

RESULTS AND DISCUSSION

RMS-deviation

To determine equilibrated-structure RMSD measurements under satisfactorily-long-range-molecular-dynamics-trajectory have much importance. We performed 200 ns production-dynamics run. The close inspection of the trajectory of the system shows important conformational changes during the initial period of last 100ns molecular dynamics run and higher values of rmsd (around 6.5 Å) in reference to starting structure was obtained which was stabilized after next 40 ns production-dynamics-run. The stereo-plot of average structure of last 100ns dynamics of the duplex is shown in fig. 3. The right side of the fig. shows the major-groove side-view of modified base-pair in the duplex, which is intercalated inside the neighbour base-pairs. RMSD for other similar sets is within 7 Å as compared to the initial frame of production-dynamics-run.

Trajectory analysis

Trajectory-analysis of base-pair parameters is important in the identification of the conformational behavior of the molecule. It is noticed that the unestablished value of some of the parameters during initial 30ns trajectory which is stabilized after 40 ns dynamics of the production-dynamics run. A significant change in base-pair-step parameters near to the modified base-pair after 30 ns to 40 ns dynamics of the production-dynamics trajectory is observed. The Slide parameters in the close vicinity of modified base-pair are shifted towards higher values which are compensated by negative values of another side of neighbor base-pairs. As an example at CG/CG step, which is above the modified base-pair hovers about 4 Å and stabilized after 20 ns and shifts towards negative values (maximum value is 7.3Å, however, minimum value is-0.63Å), while in GC/GC step, below the modified base-pair, has opposite tendency (max. 1.84 Å min.-3.463 Å) where slide parameters

hovers about-1.5 Å in range of maximum 1.84 Å to minimum-3.46 Å, and after 30 ns, it stabilizes at near to 0.1 Å, shifted towards positive values. For CG/CG step, initially, it is in positive region (min-3.5Å max. 4.9Å), but after 20 ns get stabilized near to 1.5 Å (fig. 4).

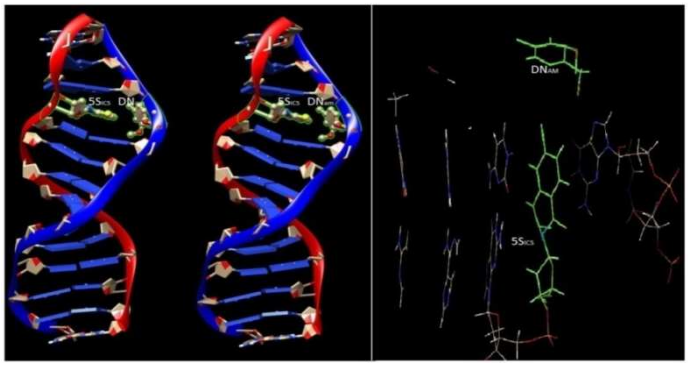

Fig. 3: Stereoview of the average structure of the molecule, green selection represents another view of the molecule

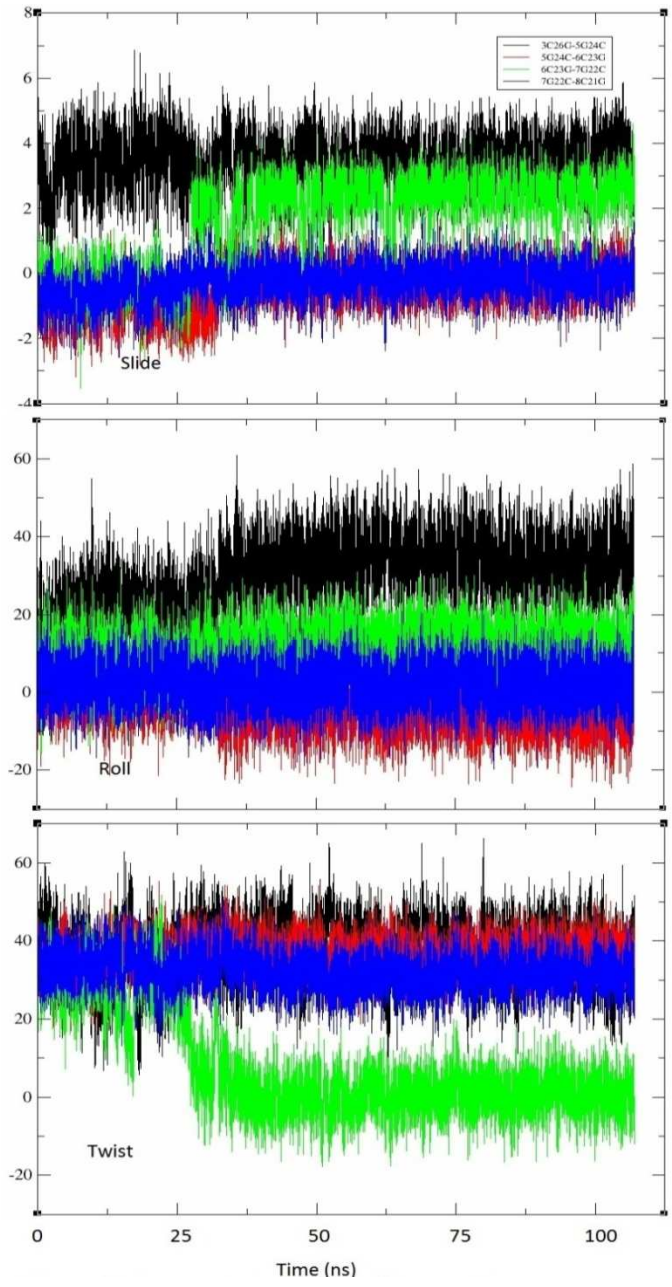

Fig. 4: The variations of slide, roll and twist during the dynamics

Slide parameters of other steps are hovers about their average values. Similarly, Propeller twist above the modified base-pair hovers in positive region and have the similar characteristic, except for CG/CG-step, where initially it hovers near to 320 and after 30 ns it dramatically shifted towards 50, the twist-parameter of different base-steps hover about the average value of the initial structure. This effect is due to larger size of modified base-pair, which needs a larger area than natural base-pairs to fit in the same diameter of the DNA duplex. To overcome steric hindrance in such condition intercalation of bases are observed in the duplex. Which is more clear in the analysis of roll for CG/CG base step, which hovers initially near to 16 °and has increasing tendency till 30 ns and after this gets stabilised near to 29 ° (average value 29.43 °). However, for GC/GC steps it stabilised in the negative region due to the similar cause as discussed above. During the MD studies of nucleic acid, trajectory-analysis of base-pair parameters is orthogonal to base-pair parameters to judge the conformational behaviour of a duplex. We observed stabilisation in these parameters after 40-ns dynamics in final trajectory (fig. 5). Propeller angle above the modified base-pair is hovering in the positive region after 30 ns dynamics, and below the modified base-pair, it is hovering in the negative region because of intercalations in base-pairs. Intercalation in bases may be confirmed by buckle angle. The modified base-pair get stabilised by modifying buckle in positive side in upper C-G base pairs, and forced towards the negative value for lower G-C base pairs, and stabilises after 20 ns dynamics run. The stabilisations of these parameters after 40-ns dynamics trajectory indicates phase transformation of the duplex which may be confirmed by the rms-deviation of the trajectory of the molecule with respect to the confirmation at 20-ns; which is low and satisfactory and get stabilized after the 39.5-ns time, and it is shown in fig. 2. The major and minor-groove-widths are important to quantify the intercalation of a modified base-pair. The major groove is highly affected above to modified base-pair (C-G) and it shrinks to about 16 Å, however, the minor-groove width of C-G is around 11 Å. Similarly, G-C base-pair, below to modified base-pair in the sequence, is equilibrated about 20 Å after 40-ns production–dynamics. The minor groove of this base-pair is about 13 Å, which clearly indicates the intercalation of modified base-pair (fig. 3), and is due to the reasons discussed above, a local-distortion is observed near the modified base-pair.

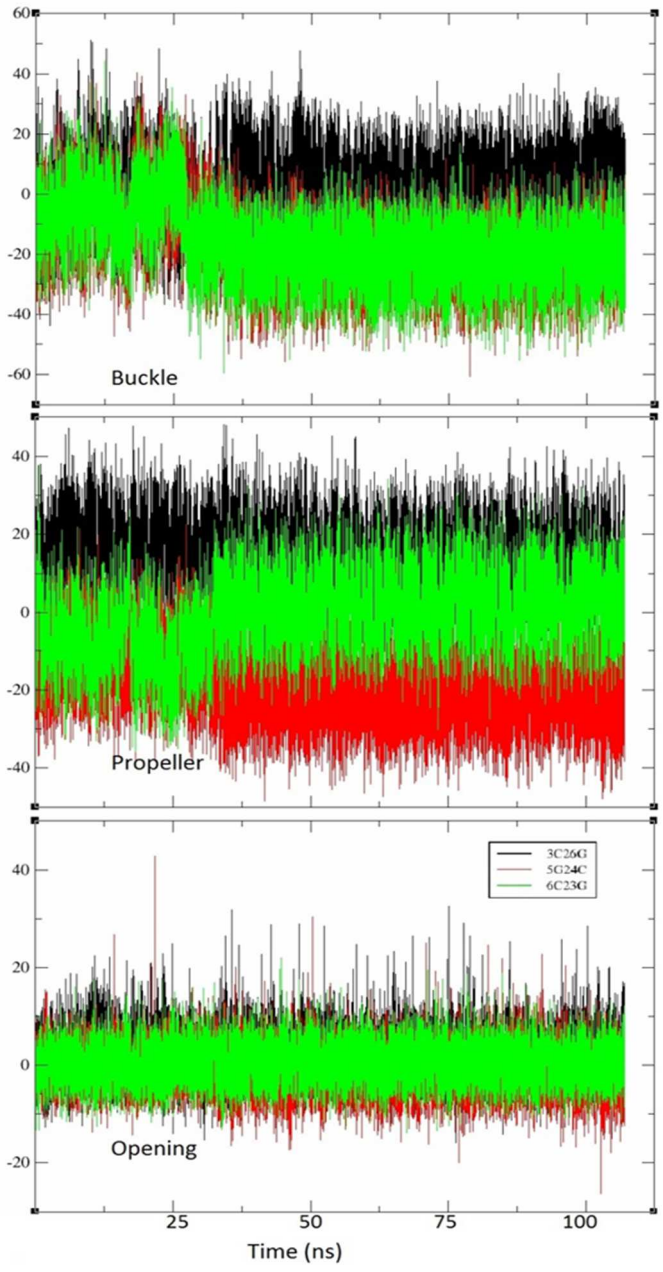

Fig. 5: Buckle, propeller and opening of neighbour base-pairs of hydrophobic base-pairs

Table 1: Local base-pair parameters modified duplex and the average value of crystal structure

S. No.	Base-pair	Buckle		Propeller		Opening	
		Modified duplex	Average value of crystal structures	Modified duplex	Average value of crystal structures	Modified duplex	Average value of crystal structures
1	G-C	-----	-7.31	------	-3.66	--	-1.69
2	A-T	-1.1	-5.99	-11.3	-15.86	-0.9	2.88
3	C-G	10.9	0.52	-14.4	-6.58	1.3	-0.24
4	C-G	11.1	-4.59	4.4	-12.27	3.7	6.04
5	A-T	-5.5	-2.79	3.5	-13.37	3.0	-0.41
6	C-G	-13.5	-1.84	-13.5	-11.49	0.2	-2.25
7	G-C	-2.0	8.44	-15.2	-23.46	2.0	2.24
8	G-C	13.1	-6.54	-12.9	-7.44	0.3	3.00
9	C-G	6.6	-0.09	1.9	1.65	2.0	1.98
10	G-C	-18.1	4.74	-1.4	-14.92	1.8	3.67
11	C-G	-17.0	10.10	-19.4	-0.36	0.1	5.88
12	DN_{AM}-$D5_{SICS}$	-85.0	21.67	26.9	-13.69	22.2	1.30
13	G-C	1.1		14.5		2.8	
14	A-T	-14.7		-13.1		6.5	
15	A-T	125.4		99.4		-13.7	
	Ave.	-3.5	1.36	0.5	-10.12	2.2	1.87

Local base-pair parameter

The average value of Shear, Stretch, Stagger, Buckle, Propeller, and Opening are 0.35 Å,-0.06 Å, 0.76 Å,-3.50, 0.50, and 3.20 respectively for the duplex. The calculations related with modified base-pairs are done manually by assuming atoms in modified nucleotides mimic to A-T base pair. We have already noted that propeller and buckle angles show significant variations during the dynamics and their average value with the average value of corresponding crystal structures [13, 31] is shown in table 1. The average value of propeller and buckle are 0.5 ° and-3.5 ° respectively, while the average value for modified-base-pair is 26.9 ° and-85 °. Local base-pair step parameters: Shift, Slide, Rise, Tilt, Roll and Twist are calculated by curve+and their average value has been found-0.18 Å, 0.3 Å, 3.36 Å, 4.7 °, and 8.6 ° respectively (table 4). The average values of these parameters for modified base-pairs-step 4(D5sics)/5(G) are-4.08 Å, 1.24 Å, and 21.4 ° respectively. The higher value of "rise" (6.2 Å) in inter-base-pair 3(C)/4(D5SICS) may be considered as a compensation-effect resulting due to restoring force of distorted-neighbour-base-steps D5SICS/G (1.24 Å). It is similar to the distortion produced by introducing irregular material of different force-constant in a part of a harmonic oscillator. Similarly Shift observed in base-step 3(C)/4(D5SICS) in positive x-direction (1.52) compensated by neighbour step 4(D5SICS)/5 (G) (-4.08 Å) in opposite direction. Slide, complementary to Shift, the important parameters to measure shifting of base-pair in the y-direction, are 6.23 Å for 3(C)/4(D5SICS) base-step and in opposite directions for neighbour base-step 4(D5SICS)/5 (G) i.e. (-2.79 Å). The higher value of Tilt and Roll, in opposite direction to each other in base-step 3/4 and 4/5, clearly manifests the accommodation of larger geometry of base-pair than W-C base-pair-geometry within the DNA diameter. The time-variation of parameters of the modified base-pair and its neighbour is observed a little during the last 50 ns dynamics. These values are calculated by Curve+, and is consistent with the value observed by 3DNA. The irregular-values of these parameters are introduced here due to the distortion produced by the geometry of the modified base-pair, which is hydrophobic in nature and doesn't have loan-pairs. And hence it doesn't support any kind of Watson-Crick type of bonding in the modified base-pair. Slide, Roll and Twist, the important parameters to decide conformation of a duplex, show more variations during initial 30-ns time-scale; which may be considered here as a conformational change of the duplex after 40-ns dynamics. The average values of these parameters for the average structure of last 100 ns dynamics are shown in table 2. We note that the average value of twist is 24.0 ° which noticeably represent stretching in the DNA-strands, particularly at the modified site. The variation in twist angles are higher in G/T and C/T base steps, and it decreases in 4(D5SICS)/5(G)-base-step by 8.4 °. Similar trends are also seen in Slide and Roll parameters in the average structure. Local base-pair helical parameters X-displacement, Y-displacement, Helical-Rise, Inclination, and Tip are shown in table 3. The average value of X-displacement is-1.01 Å, and show interesting behavior; that is positive value towards 5'-end from the modified base-pair position and negative value towards 3'-end of the strand.

Table 2: Local base-pair step parameters of the modified-duplex and average value of crystal structures

S. No.	Step	Slide		Roll		Twist	
		Modified duplex	Average value of crystal structures	Modified duplex	Average value of crystal structures	Modified duplex	Average value of crystal structures
1	GA/TC	----	-0.72	------	2.23	----	33.78
2	AC/GT	-1.12	-0.98	-5.5	7.22	40.2	28.53
3	CC/GG	0.49	-0.81	3.9	6.31	30.9	32.39
4	CA/TG	2.83	0.20	14.7	7.88	-5.3	35.81
5	AC/GT	-0.30	-0.50	-1.7	-0.40	27.5	33.32
6	CG/CG	-0.46	0.18	13.7	11.88	36.2	30.15
7	GG/CC	-1.14	-0.51	6.2	-2.42	33.2	34.00
8	GC/GC	-0.32	-0.73	1.4	6.95	30.2	29.66
9	CG/CG	1.62	-0.01	12.4	9.04	10.1	38.74
10	GC/GC	-0.55	-1.60	-4.7	10.87	33.6	25.03
11	N_{AMC}/$GD5_{sics}$	-2.79	-1.23	-31.5	6.67	8.4	32.57
12	CT/AG	6.23	-	53.3	-	38.6	-
13	TG/CA	1.05	-	8.0	-	26.8	-
13	GA/TC	-1.70	-	44.9	-	-0.1	-
	Average	0.30	-0.61	8.0	6.02	24.0	32.18

Table 3: The average value of different parameters of modified duplex

Inter base-pair parameter		Shear	Stretch	Stagger	Buckle	Propel	Opening
1	T 1-A 28	0.11	-1.11	3.45	125.4	99.4	-13.7
2	T 2-A 27	-0.23	-0.07	-0.18	-14.7	-13.1	6.5
3	C 3-G 26	0.00	-0.07	-0.36	1.1	14.5	2.8
4	**D_{NAM}4-D5$_{sics}$25**	5.15	0.86	6.03	-85.0	26.9	22.2
5	G 5-C 24	-0.04	-0.12	-0.01	-17.0	-19.4	0.1
6	C 6-G 23	-0.09	-0.05	0.23	-18.1	-1.4	1.8
7	G 7-C 22	0.06	-0.02	0.08	6.6	1.9	2.0
8	C 8-G 21	-0.05	-0.03	-0.04	13.1	-12.9	0.3
9	C 9-G 20	0.01	-0.09	-0.07	-2.0	-15.2	2.0
10	G 10-C 19	0.15	-0.01	0.16	-13.5	-13.5	0.2
11	T 11-A 18	-0.05	-0.03	0.24	-5.5	3.5	3.0
12	G 12-C 17	-0.09	-0.02	0.36	11.1	4.4	3.7
13	G 13-C 16	0.08	-0.05	0.38	10.9	-14.4	1.3
14	C 14-G 15	-0.08	-0.10	0.29	-1.1	-11.3	-0.9
Average		0.35	-0.06	0.76	-3.5	0.5	2.2

	Inter-BP	Shift	Slide	Rise	Tilt	Roll	Twist
1	T 1/T 2	1.12	-1.70	3.45	81.0	44.9	-0.1
2	T 2/C 3	0.26	1.05	3.25	2.5	8.0	26.8
3	C 3/D_{NAM} 4	1.52	6.23	5.46	-20.4	53.3	38.6
4	D_{NAM} 4/G 5	-4.08	-2.79	1.24	21.4	-31.5	8.4
5	G 5/C 6	-0.07	-0.55	3.49	-1.2	-4.7	33.6
6	C 6/G 7	-0.43	1.62	2.95	-0.8	12.4	10.1
7	G 7/C 8	-0.09	-0.32	3.25	-0.1	1.4	30.2
8	C 8/C 9	0.02	-1.14	3.64	0.7	6.2	33.2
9	C 9/G 10	-0.15	-0.46	3.54	-2.7	13.7	36.2
10	G 10/T 11	0.07	-0.30	3.26	0.4	-1.7	27.5
11	T 11/G 12	-0.29	2.83	3.06	0.7	14.7	-5.3
12	G 12/G 13	-0.60	0.49	3.48	-0.2	3.9	30.9
13	G 13/C 14	0.43	-1.12	3.67	-0.4	-5.5	40.2
Average		-0.18	0.30	3.36	4.7	8.6	24.0

Table 4: Backbone parameters of modified duplex

Strand 1		Alpha	Beta	Gamma	Delta	Epsilon	Zeta	Chi	Phase	Amplitude	Pucker
T	1	----	----	59.7	134.4	-169.1	-86.7	-74.5	141.2	41.0	C1'ex
T	2	-87.5	68.6	-175.0	132.8	-124.7	-75.9	-155.7	135.9	36.8	C1'ex
C	3	91.8	-159.2	-169.8	135.6	-108.9	-75.9	-96.2	141.4	33.3	C1'ex
D_{NAM}	4	-73.5	174.5	48.5	118.1	-84.0	77.4	-105.1	113.1	31.6	C1'ex
G	5	79.0	-175.2	-167.5	137.4	-170.0	-90.3	-133.8	157.3	27.1	C2'en
C	6	-70.1	177.0	52.3	120.0	-123.0	-81.8	-127.3	121.8	30.5	C1'ex
G	7	-88.1	151.1	49.0	122.4	-67.9	105.8	-63.4	132.6	39.0	C1'ex
C	8	85.2	-141.7	-172.4	129.4	-167.0	-86.2	-132.3	140.4	28.3	C1'ex
C	9	-72.4	174.1	50.8	117.6	-170.7	-92.7	-127.5	120.7	33.8	C1'ex
G	10	-69.2	177.5	48.3	120.6	-171.3	-90.8	-124.1	126.8	28.8	C1'ex
T	11	-68.4	174.3	52.0	121.3	-109.3	-72.8	-115.6	122.4	33.2	C1'ex
G	12	130.8	138.0	106.9	128.4	-70.2	86.5	-39.3	130.8	40.2	C1'ex
G	13	77.9	-154.6	-165.7	148.8	-174.9	-91.8	-123.5	172.7	32.9	C2'en
C	14	-73.6	179.2	53.0	119.5	----	----	-128.6	122.0	28.3	C1'ex
Strand 2											
A	28	-90.5	77.4	-173.6	111.8	----	----	-32.6	109.3	40.1	C1'ex
A	27	79.4	-154.7	-167.8	151.8	-152.2	-81.3	-111.8	174.8	33.7	C2'en
G	26	-73.3	152.9	46.0	124.7	-70.0	85.3	-33.7	127.5	40.5	C1'ex
D5$_{sics}$	25	118.1	177.5	74.6	116.2	-82.8	-58.2	-107.2	115.3	33.2	C1'ex
C	24	81.8	-118.9	179.1	130.6	-156.4	-80.1	-136.3	143.7	30.8	C1'ex
G	23	103.1	-160.0	-169.3	127.7	-80.0	123.1	-76.6	136.5	36.6	C1'ex
C	22	-68.8	173.9	52.8	126.2	-132.1	-82.0	-117.4	130.9	32.5	C1'ex
G	21	-66.5	175.0	50.0	109.7	-169.6	-88.8	-135.0	107.8	32.2	O1'en
G	20	-79.2	177.0	53.2	133.9	-174.4	-97.2	-114.5	148.3	32.9	C2'en
C	19	74.2	-142.5	-164.3	113.7	-161.3	-80.3	-142.9	102.9	23.9	O1'en
A	18	90.0	-165.3	-175.2	132.7	-66.6	90.5	-45.6	133.3	41.5	C1'ex
C	17	-70.7	171.9	46.6	130.2	-134.3	-70.5	-101.1	134.8	38.3	C1'ex
C	16	-70.5	-171.0	45.9	135.3	-167.1	-84.7	-115.5	154.0	30.5	C2'en
G	15	----	----	93.9	127.9	-163.5	-85.6	-162.6	136.1	32.0	C1'ex

Overall these values are different than corresponding values of B-DNA conformation, which was our starting-conformation in the simulation (fig. 4). The average value of Y-displacement and Inclination angle is-0.11 Å and 9.5 ° respectively, which represents

that bases are not perpendicular to the helix axis and total axis-bending observed for of duplex is about 29 °. Major and Minor groove parameters which are superlative to phosphate-phosphate distance measurement, are calculated by the method given in X3DNA, which is compatible with the method of Lavery [27]. The time variation of Major and Minor groove parameters are shown in fig. 5. The phosphate-phosphate distance in corresponding crystal structures of KlenTaq DNA polymerase, the large fragment of the type I DNA polymerase from Thermus-Aquaticus, complexes with a templating dNAM, and with or without bound-d5SICSTP, is about 11 Å which is almost similar to natural-DNA, and without polymerase it is 9.1 Å less than 0.4 Å to natural DNA. The geometrical selectivity and energy stability of DNA strands are two important key factors for entry in cellular mechanism if unnatural bonds mimic to natural bonds [10]. Their elective properties produce selectivity of DNA polymerase in replication, which is the first challenge to acceptance of foreign molecule in nucleic acid function. After that, nucleic acids have to retain the unnatural base pairs during central dogma for very large time domain. The variations observed between natural/natural and natural/modified base-step and vice-versa clearly support the experimental results. The average value of time variation of backbone parameters (alpha, beta, gamma, delta, epsilon zeta and chi) are represented in table 3. The pucker of strands is in the range of C1'-exo to C2'-endo. Therefore it may be concluded that DNA parameters are destabilised near the modified base-pair and affect over the entire strand. Similar observations are found for other sets of the complex. It is realized that these parameters represent a mixed character of DNA conformation, however, in the crystal structure, the position of DNAM in the templet is stabilised by an ionic interaction between its phosphate and ARG residue of the polymerase. The stability of duplex greatly depends on the confirmation of polymerase and it appears that polymerase provides unique stabilizing interactions to the two type of intercalated structures at the primer terminus. Water and ion distribution around duplex molecule were calculated using the CPPTRAJ module of AMBER 14 [25, 32, 33]. The number of water molecules forming the hydrogen bonds with the duplex was calculated at various intervals during the dynamics. At any given time, some peculiar water molecules, which are present in a specific pocket for a certain length of time, forms a hydrogen bond with a specific group of atoms. In trajectory-analysis, we have found 406 water molecules within the first layer and 691 water molecules in the second layer. The time duration for which the specific water molecule is forming a hydrogen bond with the specific group of atoms can be ascertained by looking into the structure at small intervals of time.

CONCLUSION

During the production-dynamics simulation the average value of fluctuation shown by heavy atom is minimum (about 19 Å), however, the average values of fluctuation for hydrogen atoms are about 26 Å. The fluctuations for sodium ions during production-dynamics range is found to be a slightly higher value (∼27.5 Å), due to the obvious reason that they are not part of the duplex and allowed to do free-motion in solution. A high distortion in duplex strand has been observed if we place modified base-pair in first and second place of the sequence from either 5' or 3' end of the duplex, not included in this study. However, we get a stabilized structure of such type of duplex if we place modified base-pair after the third place in the strand. On the basis of above discussions, It may be concluded that distortion produced by modified-base-pair in the overall structure of duplex is local. The confirmation of such type of duplex is mixed and maintains the W-C type of integrity [34].

Simulations were run on HP workstation, in our laboratory at DDU Gorakhpur University, Gorakhpur. Some calculations have been completed at the super computational facility (BRAF) of CDAC, Pune.

ACKNOWLEDGEMENT

Authors are thankful to UGC, New Delhi for financial support and acknowledging CDAC, Pune for providing the computational facility.

AUTHOR CONTRIBUTION

All the work have been carried out by me

CONFLICTS OF INTERESTS

Author has none to declare

REFERENCES

1. Zhang Y, Lamb BM, Feldman AW, Zhou AX, Lavergne T, Li L, *et al.* A semisynthetic organism engineered for the stable expansion of the genetic alphabet. Proc Natl Acad Sci USA 2017;114:1317–22.
2. Ashwood B, Pollum M, Crespo-Hernandez CE. Can a six-letter alphabet increase the likelihood of photochemical assault to the genetic code? Chemistry (Easton) 2016;22:16648–56.
3. Schweitzer BA, Kool ET. Hydrophobic, non-hydrogen-bonding bases and base pairs in DNA. J Am Chem Soc 1995;117:1863–72.
4. Li Z, Lavergne T, Malyshev DA, Zimmermann J, Adhikary R, Dhami K, *et al.* Site-specifically arraying small molecules or proteins on DNA using an expanded genetic alphabet. Chemistry (Easton) 2013;19:14205–9.
5. Wang W, Sheng X, Zhang S, Huang F, Sun C, Liu J, *et al.* Theoretical characterization of the conformational features of unnatural oligonucleotides containing a six nucleotide genetic alphabet. Phys Chem Chem Phys 2016;18:28492–501.
6. Chaplin M. Do we underestimate the importance of water in cell biology? Nat Rev Mol Cell Biol 2006;7:861–6.
7. Malyshev DA, Dhami K, Lavergne T, Chen T, Dai N, Foster JM, *et al.* A semi-synthetic organism with an expanded genetic alphabet. Nature 2014;509:385–8.
8. Betz K, Malyshev DA, Lavergne T, Welte W, Diederichs K, Dwyer TJ, *et al.* KlenTaq polymerase replicates unnatural base pairs by including a Watson-Crick geometry. Nat Chem Biol 2012;8:612–4.
9. Malyshev DA, Pfaff DA, Ippoliti SI, Hwang GT, Dwyer TJ, Romesberg FE. Solution structure, mechanism of replication, and optimization of an unnatural base pair. Chemistry (Easton) 2010;16:12650–9.
10. Lavergne T, Malyshev DA, Romesberg FE. Major groove substituents and polymerase recognition of a class of predominantly hydrophobic unnatural base pairs. Chem A Eur J 2012;18:1231–9.
11. Yamashige R, Kimoto M, Takezawa Y, Sato A, Mitsui T, Yokoyama S, *et al.* {Highly specific unnatural base pair systems as a third base pair for PCR amplification. Nucleic Acids Res 2012;40:2793–806.
12. Dixit SB, Beveridge DL, Case DA, Cheatham TE, Giudice E, Lankas F, *et al.* {Molecular dynamics simulations of the 136 unique tetranucleotide sequences of DNA oligonucleotides. II: sequence context effects on the dynamical structures of the 10 unique dinucleotide steps. Biophys J 2005;89:3721–40.
13. Malyshev DA, Pfaff DA, Ippoliti SI, Hwang GT, Dwyer TJ, Romesberg FE. {Solution structure, mechanism of replication, and optimization of an unnatural base pair. Chemistry (Easton) 2010;16:12650–9.
14. Dutta Dubey K, Tiwari RK, Ojha RP. Recent advances in protein-ligand interactions: Molecular dynamics simulations and binding free energy. Curr Comput Aided Drug Des 2013;9:618-31.
15. Ojha RP, Tiwari RK. Triplex hydration: nanosecond molecular dynamics simulation of the solvated triplex formed by mixed sequences. Nucleic Acids Res 2003;31:6373-80.
16. Ojha RP, Tiwari RK. Molecular dynamics simulation study of DNA triplex formed by mixed sequences in solution. J Biomol Struct Dyn 2002;20:107-26.
17. Muttaqin F, Fakih T, Muhammad H. Molecular docking, molecular dynamics, and *in silico* toxicity prediction studies of coumarin, n-oxalylglycine, organoselenium, organosulfur, and pyridine derivatives as histone lysine demethylase inhibitors. Asian J Pharm Clin Res 2017;10:212-5.
18. Kumar AVS, KR. Structural and functional impact of g2032r mutation in ros1–a theoretical perspective. Asian J Pharm Clin Res 2017;10:339–44.
19. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, *et al.* UCSF chimera—a visualization system for exploratory research and analysis. J Comput Chem 2004;25:1605–12.

20. Blanchet C, Pasi M, Zakrzewska K, Lavery R. CURVES+web server for analyzing and visualizing the helical, backbone and groove parameters of nucleic acid structures. Nucleic Acids Res 2011;39:68–73.
21. Arnott S, Wonacott AJ. The refinement of the crystal and molecular structures of polymers using X-ray data and stereochemical constraints. Polymer (Guildf) [Internet] 1966;7:157–66.
22. Pandit, Rakesh KR, GUPTA, Dinesh, Mukherjee TK. Identification of potential salmonella typhi beta-lactamase tem 1 inhibitors using peptidomimetics, virtual screening, and molecular dynamics simulations. Int J Pharm Pharm Sci 2018;10:91–6.
23. Jo S, Cheng X, Lee J, Kim S, Park SJ, Patel DS, *et al.* Charmm-GUI 10 y for biomolecular modelling and simulation. J Comput Chem 2017;38:1114–24.
24. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, *et al.* Gaussian~09 Revision E.01; 2016.
25. Lee J, Cheng X, Swails JM, Yeom MS, Eastman PK, Lemkul JA, *et al.* Charmm-GUI input generator for namd, gromacs, amber, open MM, and charmm/OpenMM simulations using the CHARMM36 additive force field. J Chem Theory Comput 2016;12:405–13.
26. Cieplak P, Cornell WD, Bayly C, Kollman PA. Application of the multimolecule and multi-conformational RESP methodology to biopolymers: charge derivation for DNA, RNA, and proteins. J Comput Chem 1995;16:1357–77.
27. Blanchet C, Pasi M, Zakrzewska K, Lavery R. CURVES+web server for analyzing and visualizing the helical, backbone and groove parameters of nucleic acid structures. Nucleic Acids Res 2011;39:68–73.
28. Wang Y, Bryant SH, Cheng T, Wang J, Gindulyte A, Shoemaker BA, *et al.* {PubChem BioAssay: 2017 update. Nucleic Acids Res 2017;45:D955–63.
29. Yang Z, Lasker K, Schneidman-Duhovny D, Webb B, Huang CC, Pettersen EF, *et al.* UCSF chimera, MODELLER, and IMP: an integrated modelling system. J Struct Biol 2012;179:269-78.
30. Beccuti M, Carrara M, Cordero F, Lazzarato F, Donatelli S, Nadalin F, *et al.* Chimera: a bioconductor package for secondary analysis of fusion products. Bioinformatics 2014;30:3556–7.
31. Betz K, Malyshev DA, Lavergne T, Welte W, Diederichs K, Dwyer TJ, *et al.* KlenTaq polymerase replicates unnatural base pairs by inducing a watson-crick geometry. Nat Chem Biol 2012;8:612–4.
32. Case DA, Cheatham TE, Darden T, Gohlke H, Luo R, Merz KM, *et al.* The amber biomolecular simulation programs. J Comput Chem 2005;26:1668–88.
33. Dubey KD, Tiwari RK, Ojha RP. Recent advances in protein-ligand interactions: molecular dynamics simulations and binding free energy. Curr Comput Aided Drug Des 2013;9:518–31.
34. Tiwari RKT, Ojha RP. DNA Triplex. Lambeert: conformation and stability of mixed DNA triplex; 2012. p. 25-30.