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Structure and dynamics of a DNA duplex containing single α-anomeric deoxyadenosine residue★✪

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Structure and dynamics of an undecamer DNA duplex containing a single α-anomeric deoxyadenosine residue placed in opposition to a thymidine unit have been studied using simulation of molecular dynamics in aqueous solution. Despite several noticeable deviations from the B-DNA duplex structure caused by the anomerisation, such as: West type puckering of the α-anomeric sugar, disrupted base stacking pattern and unstable duplex bending, the formation of a non-classical α-dA–T pair was observed. A novel way of visual presentation of trajectory data allowing high throughput screening of the conformational parameters is presented.

Nearly all nucleosides found in nature, in particular those constituting nucleic acids, are β-anomers, i.e. the heterocyclic base is located at the C5′ side of the (deoxy)ribose ring (see Fig. 1).

Only a few cases of α-anomeric nucleoside derivatives are known and they are consituents of a number of cofactors and similar small molecules found in bacteria, the most notable being vitamin B_{12} [1]. No such nucleosides have been identified in natural nucleic acids. Despite this, the question of the influence of an α-anomeric residue on the local and overall nucleic acid duplex structure is of practical importance, for instance when designing novel fluorescent probes, e.g. α-1,N^6-ethenodeoxyadenosine [2]. Moreover, knowing the answer to this question could help us understand the structural phenomena underlying genetic processes and also the reasons why nature uses only β-anomers to build nucleic acids.

α-Deoxyoligonucleotides are capable of forming duplexes, which has already been proved using Dreiding models in the seventies [3] and later, also experimentally [4]. It was found that α-deoxyribonucleotide tracts form typical Wat-

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Abbreviations: MD, molecular dynamics; α-dA, α-deoxyadenosine; PME, particle-mesh Ewald (a summation method); r.m.s.d., root mean square displacement.

son–Crick base pairs within antiparallel duplexes (consisting of two α-oligodeoxynucleotides) or parallel duplexes (α-oligodeoxynucleotides with β-oligodeoxynucleotides). α-Oligodeoxynucleotide segments form such parallel duplexes also when inserted into regular antiparallel DNA duplexes and linked by 3′–3′ and 5′–5′ phosphodiester bonds [5, 6]. Parallel heteroduplexes of this type are resistant to cleavage by cell nucleases [7] and they block the activity of reverse transcriptase [8]. These properties focused the interest on α-anomeric oligonucleotides as potential antisense antiviral chemotherapeutics [9, 10]. Analogous pairing properties with natural DNA are shown by α-oligoribonucleotides [11]. Oligomers composed of α-deoxynucleotides can also form triplexes with all-β B-DNA [12].

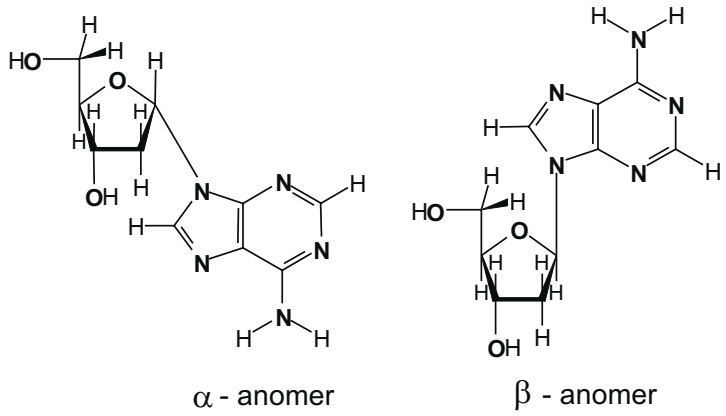

Figure 1. Anomers of deoxyadenosine

The question of the possible influence of a single α-nucleotide inserted into a regular oligonucleotide strand has not been exhaustively studied so far. Only two papers related to this problem have been published until now. The properties of 9 bp DNA duplexes, where an α-deoxyadenosine residue was placed in the middle base pair were studied by ultraviolet and circular dichroism spectral analysis [13]. Such a modification can be induced by free hydroxyl radicals in DNA under anaerobic conditions, e.g. due to ionising radiation, and it blocks DNA synthesis. The paper also presents the results of *in vacuo* molecular mechanics (geometry optimisation) experiments which suggest that the influence of such a modification on the global duplex structure is negligible, and formation of an α-dA–T pair was postulated. Unfortunately, this study did not include a molecular dynamics simulation experiment which could help evaluate the actual conformational stability of the model. In another paper, the molecular dynamics of four DNA triplexes is presented, each one containing a modified nucleoside: α- or β-anomer of 2-aminopyridine deoxyriboside in one of the strands [14]. Also in this case no important structural deviations were identified, the simulation results analysis, however, was limited to an image of the modified region of the molecule showing hydrogen bonds. Some degree of conformational instability of triplexes containing the α-anomeric residue could also be deduced from the r.m.s.d. analysis of atomic positions.

Recently, we reported on the structural properties of DNA duplexes modified with a single α-1,N^6-ethenodeoxyadenosine residue using methods of time-resolved fluorescence and thermodynamics [2]. Both methods suggested a much lower degree of destabilisation of such duplexes than expected. As it was impossible to obtain good crystals of such DNA duplexes, we employed the method of molecular dynamics simulation to gain a better insight into the conformational dynamics of those molecules. Surprisingly, the formation of an α-εdA–dG base pair was observed.

This prompted us to return to the basic problem concerning the influence of single α-anomeric nucleoside residues on DNA structure. Our preliminary *in vacuo* and early *in aqua* simulations produced severely distorted molecules, but in the

case of duplexes containing α-anomeric residues that distortion was much more expressed, severe axis bending being the most striking feature. Although a major part of those effects was certainly due to the deficiencies of the early simulation protocols, we still presumed that some degree of axis bending might result from the modification. To test this hypothesis, we decided to employ the advanced parcticle-mesh Ewald *in aqua* molecular dynamics simulation method. Here we present our results of molecular dynamics experiments performed on DNA duplexes of the sequence d(CTCCTXTCCCT):d(AGGGATAGGAG), where X stands for α-deoxyadenosine or deoxyadenosine (reference experiment). Two 1 ns long simulations were conducted at 300 K for the α-anomeric model and one for the reference molecule. The structural properties of the DNA models are presented using a new approach which allows compact visualisation of the variability of parameters over the entire molecular dynamics trajectory.

MATERIALS AND METHODS

Calculations. The initial structures were built as canonical B-DNA duplexes using the procedures provided in the AMBER 4.1 package [15]. The α-dA residue was defined according to the AMBER 4.1 model definition standards. Using the Gaussian 94 [16] and Resp [17] programs we have confirmed that α-anomerisation does not significantly affect the distribution of charges in the nucleoside. The differences in partial charges calculated for the atoms of the anomerisation center neighborhood in the α- and β-deoxyadenosine never exceeded 0.03. Hence, given that the standard charge distribution available in the force field was obtained with more precise algorithms, we decided to use the latter set of charges for our simulations.

To the model molecules were added 20 sodium counterions (3.5 Å from the phosphate centres) and they were solvated in a rectangular PBC box with a 10 Å thick water layer in all dimensions around the molecule (Edit module applied). The box dimensions were thus 62.1 Å × 45.6 Å × 45.3 Å, and the number of water molecules in the simulated system was 3973. Simulations were carried out using the Sander program (part of the AMBER 4.1 package) run on a CRAY J-916 machine in the Poznań Supercomputing and Networking Center. The protocol for the simulations followed the procedure applied by Cheatham & Kollman [18] and consisted of an equilibration procedure (alternating energy minimisation and short molecular dynamics runs with gradually relieved constraints on the DNA molecule) and 1 ns of unrestrained molecular dynamics at 300 K with long-range electrostatic interactions treated using the particle-mesh Ewald summation method. The SHAKE algorithm for maintaining the X–H atomic distances was used and the time-step for the Newtonian equations integration was set to 2 fs.

Trajectory data analysis. The resultant trajectories from all simulations were analysed and visualised on an Iris Indigo^2 workstation using the InsightII software (MSI), the CURVES 5.1 program [19] and our own procedures.

Base stacking. For the evaluation of the maintaining of stacking interactions between neighbour nucleobases in a strand, we used a parameter proposed by us earlier [20] calculated using the following formula:

$$S = D_1 + D_2 + D_3 + 2\,(|D_1-D_2|+|D_2-D_3|+|D_1-D_3|) - 15.2$$

including distances between the following atoms of subsequent residues: D_1 — N9 (purines) and N1 (pyrimidines); D_2 — N7 (purines) and C5 (pyrimidines); D_3 — C5 (purines) and N3 (pyrimidines).

Values close to zero indicate that the bases are aligned in accordance with the canonical B-DNA conformation. Values higher than 3–5 Å are observed when nucleobases lose their parallel alignment or stand aside, which results in the loss of stacking interactions in the respective region of the strand.

Duplex axis bending. There is no commonly accepted parameter for nucleic acid duplex axis bending, although several approaches have been proposed [21, 22]. Our approach is based on the

axis measurements performed by the CURVES 5.1 program [19]. The actual angle of local axis bending was calculated (three subsequent points of the axis taken into account at each step for the angle definition) and the overall bending was represented in two ways: (1) as the axis end–centre–end angle and (2) as the relative shortening (in percent, measured as 1 minus the ratio of axis end–to–end distance to its path length). Since the anomerisation severely distorts the local base pair coordinates system implemented in CURVES 5.1, it was necessary to redefine it by changing the names of the C4 and C8 atoms of the modified residue and ignoring that residue in global axis calculation.

Visualisation of trajectory data. Here we propose a novel way of presenting trajectory data allowing high throughput screening of the conformational parameters prior to their detailed analysis. We display the data in a form resembling electrophoresis gel patterns, as the parameter value is represented in the grey scale and particular "lanes" represent its values for the residues (or base pairs) within the nucleic acid strand (or duplex). This approach allows quick identification of those areas within the strand (duplex), as well as periods within the simulation history, where an irregularity might occur. The customary presentation of numerous graphs, on the other hand, forces the reader to examine them very carefully to extract and identify this sort of information.

RESULTS AND DISCUSSION

Detailed conformational analysis of the three 1 ns trajectories allows us to evaluate several interesting features of the DNA molecules studied.

In a canonical B-DNA structure, the pseudorotation angle (used to evaluate the sugar puckering) ranges between 120–180° and such values were generally maintained by the greater part of the modelled molecule. The pseudorotation angle of the deoxyribose ring of the α-anomerised nucleoside had a tendency to adopt values from

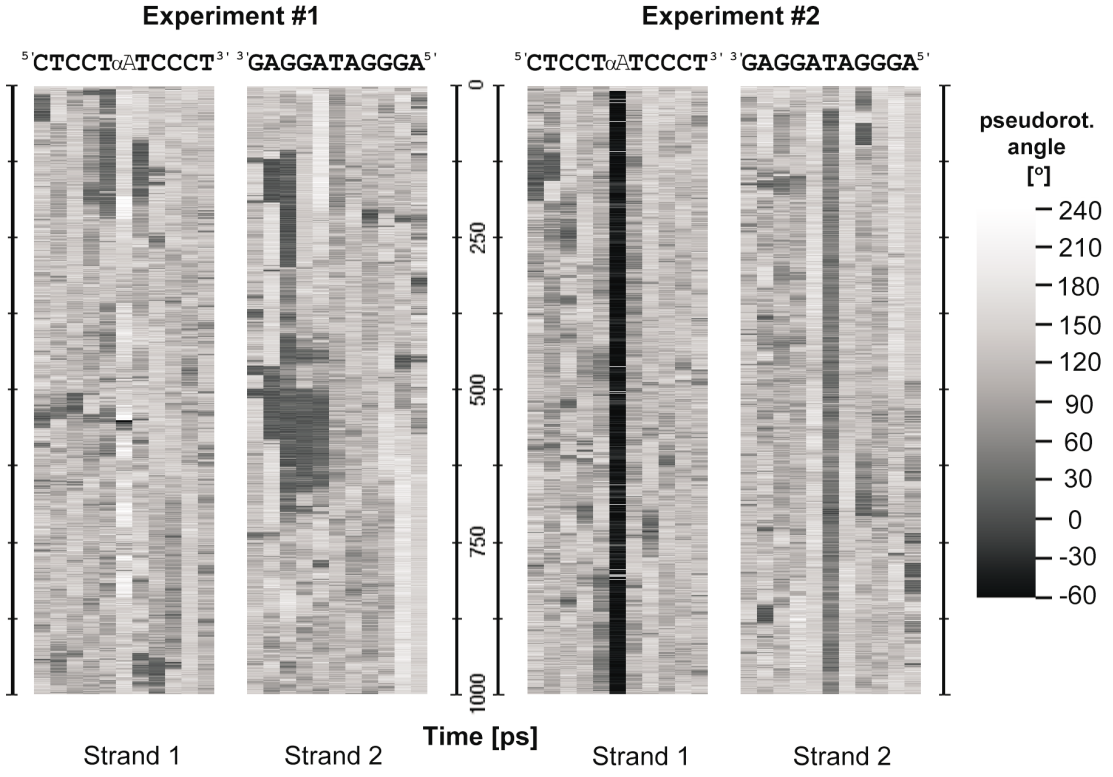

Figure 2. Diagram of pseudorotation parameter calculated for trajectories No. 1 and No. 2 for both modified and unmodified strands.

Parameter values are represented by the gray scale level; values exceeding maximum shown in white and below minimum in black.

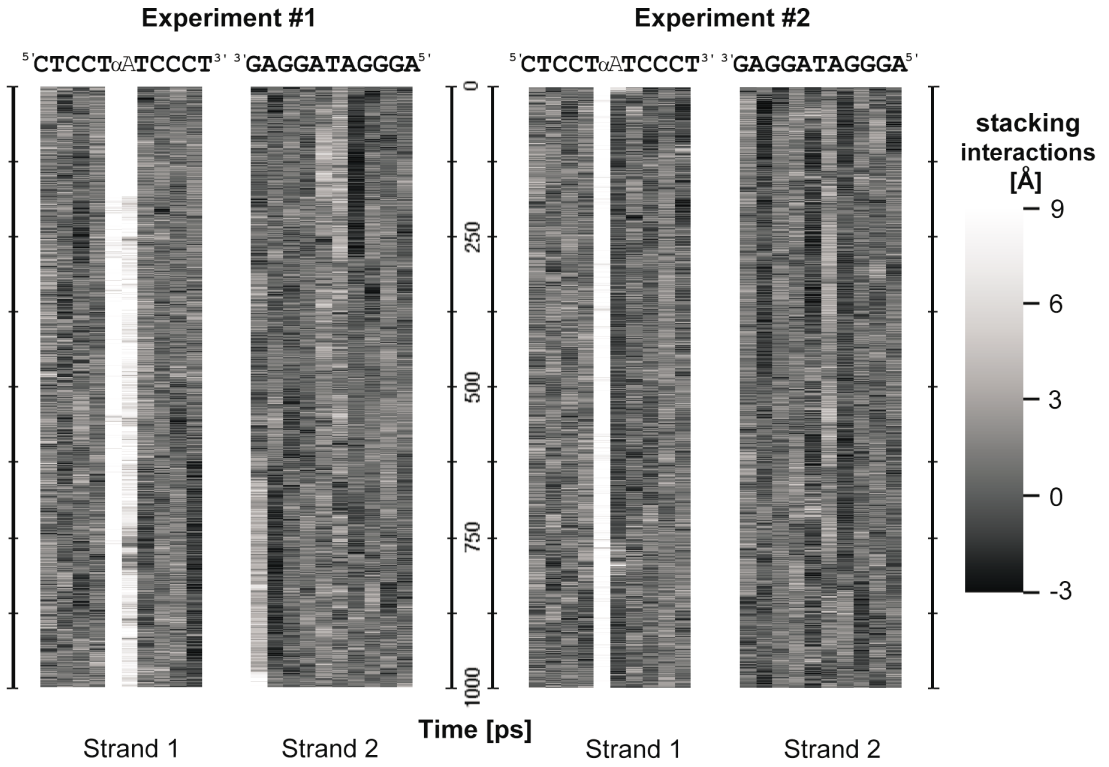

Figure 3. Diagram of stacking parameter (as defined in Fig. 2) calculated for trajectories No. 1 and No. 2 for both modified and unmodified strands.

the "West" range of the pseudorotation circle (e.g. O4′-*exo* or C1′-*endo* conformations). In simulation No. 2 this is particularly well visible (see Fig. 2) and C1′-*endo* sugar puckering is observed very of-

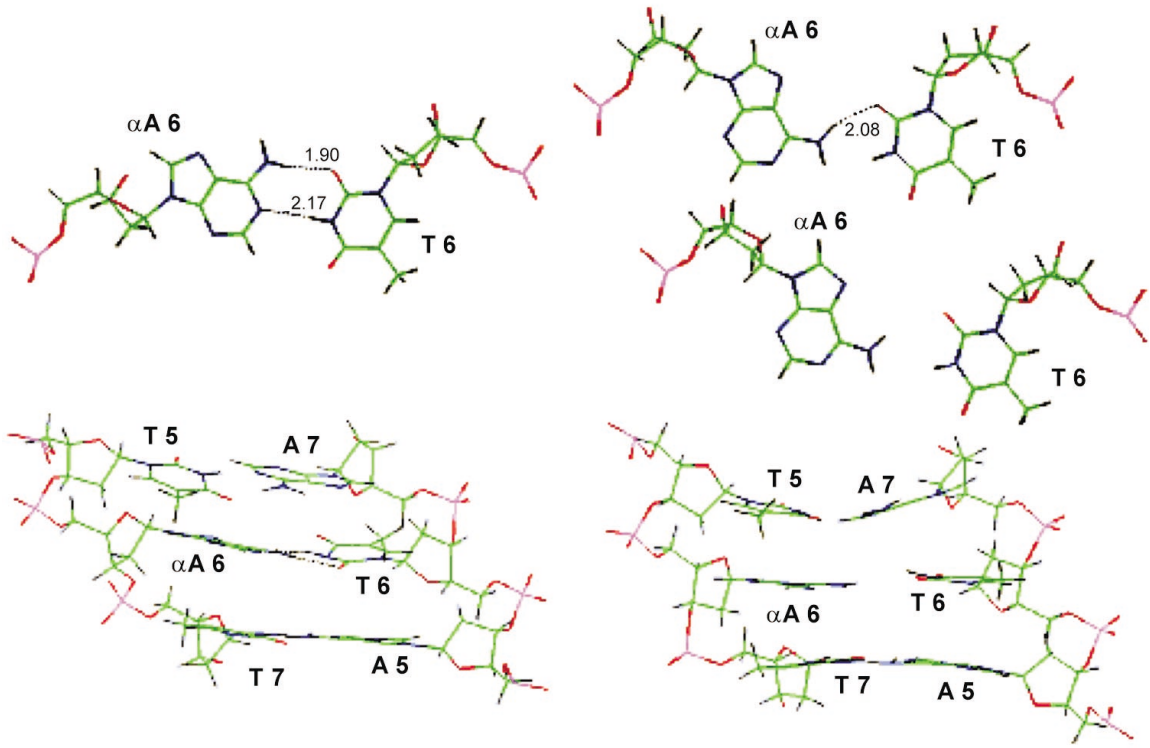

Figure 4. Typical conformations of the modified region in the last 200 ps of simulation No. 1 (left) and No. 2 (right).

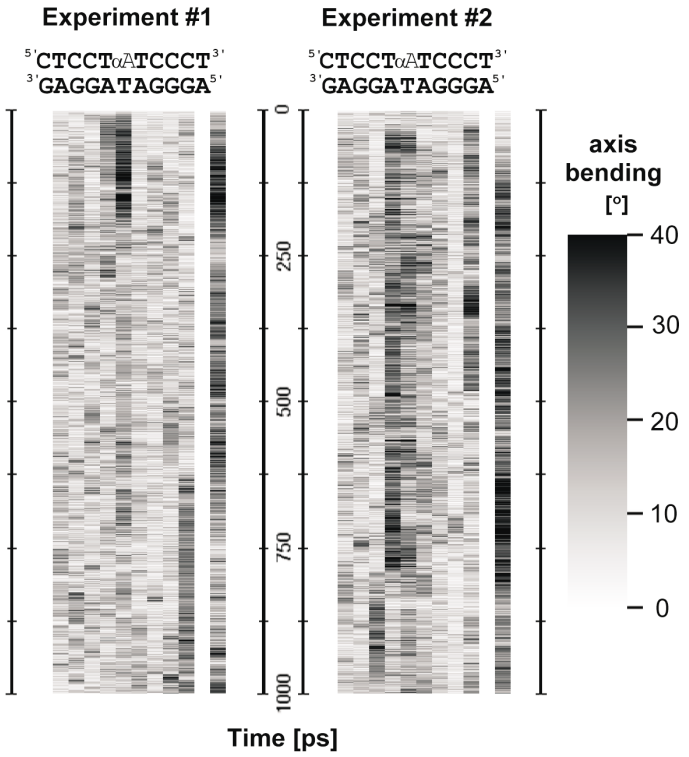

Figure 5. Diagram of local duplex bending calculated as defined in Methods for trajectories No. 1 and No. 2.

The single lane represents the end–to–end axis angle.

ten. An average value of –50° was found for the pseudorotation angle for the final 200 ps of that simulation. As the "West" values are out of the standard linear pseudorotation angle scale (–60°–240°) used in Fig. 2, the colours representing them are either black (below range) or white (above range) and the point of discontinuity (black to white change) is at 270° (= –90°). A similar effect of unusual pseudorotation angle values was found for the α-εdA residue [2]. The nucleosides located in the neighbourhood of the modification appear to be more destabilised and more often change their conformation to C3′-*endo* (i.e. puckering typical for RNA residues) than residues located farther from the α-anomer site (see Fig. 2).

The α-anomerisation causes a significant difficulty to the residue involved to adapt to the regular, base stacked alignment. This concerns the stacking of the modified residue with both its neighbours, although in simulation No. 2 we could observe that stacking with the 3′-neighbouring thymine was favoured (see Fig. 3), despite the fact that the thymine stacking properties are generally known to be low.

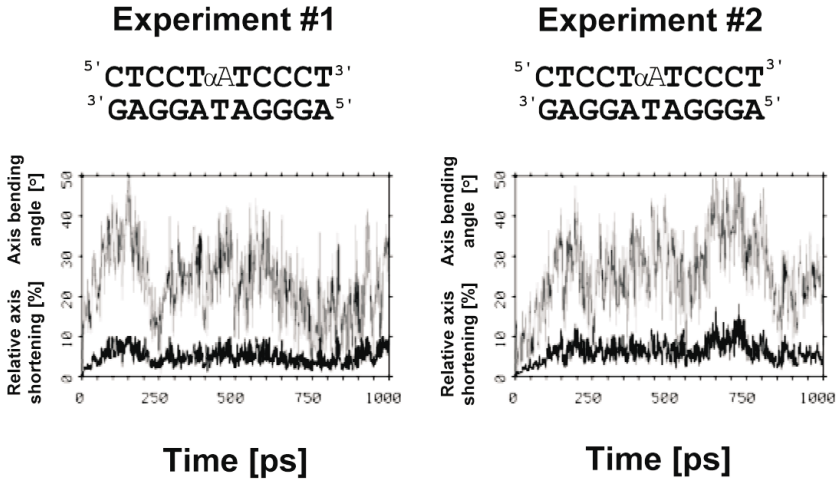

Figure 6. Graphs of global duplex bending calculated as defined in Methods for trajectory No. 1 and No. 2.

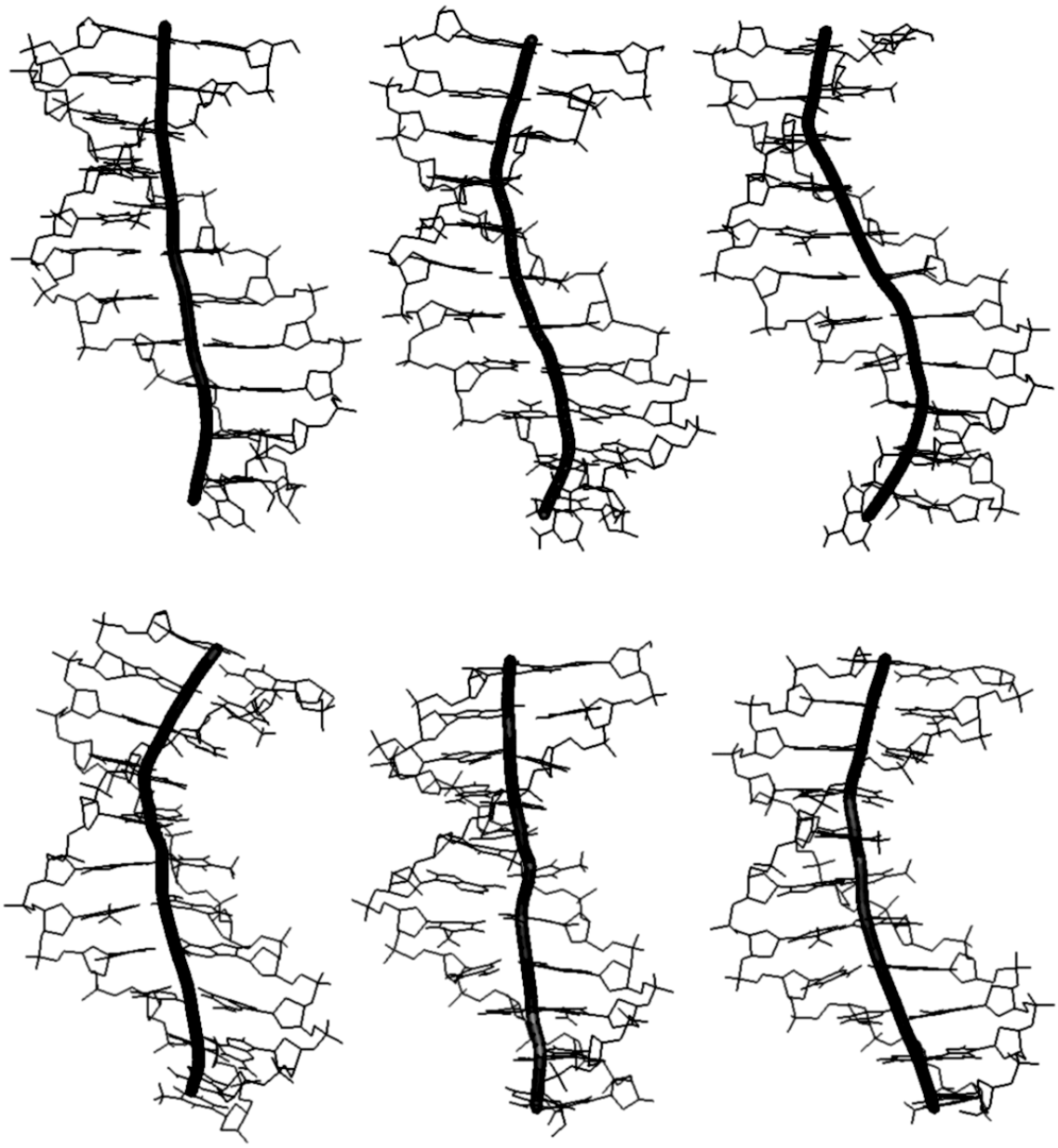

Figure 7. Images (snapshots) of typical global duplex conformations selected from the last 200 ps of simulation No. 1 (top) and No. 2 (bottom).

Duplex axis displayed as thick line.

For the unmodified reference molecule, no significant destabilisation of deoxyribose puckering or base stacking was observed.

Interestingly, the two simulations of the α-anomer-modified model brought significantly different results in regard to the alignment of the bases in the modified pair. In simulation No. 1, the bases tended to mimic the classical Watson–Crick pair normally formed by standard β-anomeric residues and two stable hydrogen bonds were observed. On the other hand, in simulation No. 2 the alignment of both bases involved did not allow for any effective hydrogen bonding, only in some periods of simulation one hydrogen bond was formed. We have identified typical conformations of the central region of the simulated model. For simulation No. 1, one general alignment of the bases was found (see Fig. 4, left) while in simulation No. 2 at least two typical conformations could be identified (Fig. 4, right).

In the unmodified duplex all base pairs were stable, except for the terminal dA–T pair which

broke in the later part of the unrestrained MD simulation. A similar base pair disruption took place in the last part of simulation No. 1 for the modified model.

Surprisingly, no major bending or kinking could be identified in any of the simulated model duplexes. Still, the duplex axis course was found to be less stable in the α-anomerised model than in the reference molecule (see Figs. 5 and 6).

Typical global conformations of the modified duplex selected from both simulations are presented in Fig. 7. In simulation No. 1, very unstable values of the helical parameters tilt, roll and twist were observed. As indicated, no duplex axis bending could be identified.

The observations presented above clearly suggest that an α-anomeric residue inserted into a DNA duplex has a destabilising effect mostly on its nearest neighbours, particularly in respect to sugar puckering and base stacking. Any bending tendency, if it occurs, is not well expressed. It might be, perhaps, deduced from the observed competition between effective base stacking of the modified residue and its stable alignment within the pair. It can be indicated that, in particular, the effective hydrogen bonding in the α-dA–T system able to mimic the classical dA–T pair, forces the modified residue to adopt a conformation which weakens its stacking capability. This is in contrast to the classical all-β DNA duplex structure where both types of interactions (stacking and hydrogen bonding) cooperate in stabilising its conformation. Nevertheless, the experiment confirms the possibility of base pair formation in the α-dA–T system using the same hydrogen bonding atoms as in the canonical pair. This adaptive ability of the duplex structure modified with an α-anomeric residue might account for the generally low efficiency of α-anomer based antiviral chemotherapeutics [9]. On the other hand, such molecules, although not very severely distorted, might still be too strongly destabilised to take part in genetic processes effectively. This feature could have disabled their usage in natural processes at a very early stage of the evolution of life, thus leaving the nucleic acids world uniformly β-anomeric.

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