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COMPARATIVE ANALYSIS OF MOLECULAR MECHANISMS OF RELAXATION OF DNA BENDING DEFORMATION

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Comparison of relaxation mechanisms of bending DNA deformation was performed by molecular dynamics method for two types of bending DNA from complex with 434 repressor and purine repressor. Comparative analysis of relaxation dynamics of local structure parameters characterizing DNA structure on dinucleotide level and parameters characterizing DNA structure as whole was performed. Obtained data shows that relaxation takes place in stages and each stage is characterized by similar time intervals for two bending DNA types. It is shown that character of relaxation in the initial stages of dynamics depends on the type of DNA bending deformation: for smooth curve linear changes in parameters are peculiar; for localized sharp curve changes in parameters occurs abruptly.
KEY WORDS: DNA, molecular dynamics, protein-nucleic acids interactions, structure deformations, relaxation mechanisms.

СРАВНИТЕЛЬНЫЙ АНАЛИЗ МОЛЕКУЛЯРНЫХ МЕХАНИЗМОВ РЕЛАКСАЦИИ ИЗГИБНЫХ ДЕФОРМАЦИЙ ДНК

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Сравнение механизмов релаксации изгибных деформаций ДНК было выполнено методом молекулярной динамики для двух типов изгибов ДНК, а именно, из комплекса с 434 репрессором и пуриновым репрессором. Был выполнен сравнительный анализ динамики релаксации локальных структурных параметров, характеризующих структуру ДНК на уровне динуклеотидного контакта и параметров, которые описывают структуру ДНК в целом. Полученные данные свидетельствуют, что релаксация проходит поэтапно и каждый этап характеризуется одинаковыми временными интервалами для двух типов изгиба ДНК. Показано, что характер релаксации на начальных стадиях динамики зависит от типа изгибной деформации ДНК: для плавного изгиба свойственно линейное изменение параметров; для резкого локализированного изгиба изменение параметров происходит скачкообразно.
КЛЮЧЕВЫЕ СЛОВА: ДНК, молекулярная динамика, белок-нуклеиновые взаимодействия, структурные деформации, механизм релаксации.

ПОРІВНЯЛЬНИЙ АНАЛІЗ МОЛЕКУЛЯРНИХ МЕХАНІЗМІВ РЕЛАКСАЦІЇ ВИГИБНИХ ДЕФОРМАЦІЙ ДНК

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Порівняння механізмів релаксації вигибних деформацій ДНК було виконано методом молекулярної динаміки для двох типів вигибів ДНК, а саме, з комплексу 434 репресору і пуринового репресору. Було виконано порівняльний аналіз динаміки релаксації локальних структурних параметрів, які характеризують структуру ДНК на рівні динуклеотидного контакту та параметрів, які описують структуру ДНК в цілому. Отримані дані засвідчують, що релаксація проходить поетапно і кожний етап характеризується однаковими часовими інтервалами для двох типів вигибу ДНК. Показано, що характер релаксації на початкових стадіях динаміки залежить від типу вигибної деформації ДНК: для плавного вигибу властива лінійна зміна параметрів; для різкого локалізованого вигибу зміна параметрів відбувається стрибкоподібно.
КЛЮЧЕВІ СЛОВА: ДНК, молекулярна динаміка, білок-нуклеїнові взаємодії, структурні деформації, механізм релаксації.

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Structural deformability of DNA is an important mechanism of indirect recognition in protein-nucleic acid complexes. Since energy costs for the emergence of deformations vary for different sequences, the ability to change the configuration depends on the sequence. Protein uses this property for the recognition of the native DNA sequence, and this is the mechanism of indirect sequence recognition [1]. Protein-nucleic acid complexes can serve as the kind of such indirect recognition mechanism where interaction with the protein leads to the bending deformations of DNA.

Fig.1 DNA from complex 434 repressor – DNA (434 Rep-DNA) (a) and DNA from complex purine repressor – DNA (PurR-DNA) (b). Arrows show the level of DNA sequence bending, place of interaction with protein is encircled.

The degree of DNA bending can be minor, while in some complex interactions with the protein leads to the significant bending of the DNA double helix. Sharp DNA curves are characteristic for interaction with protein in the minor groove [2]. Such deformations are often associated with changes in the width and depth of grooves, as well as the restructuring of the sugar-phosphate backbone, i.e. switching package sugar and change the equilibrium value of the dihedral angles [3, 4].

Nowadays sequence-specificity of DNA [5, 6] and the mechanism of direct recognition in the major groove are well studied. Dynamics of bend formation in the minor groove, caused by interactions with the protein remains obscure. In this study we investigated and compared the mechanisms of relaxation of DNA bending deformations including different bending types during interaction in minor groove.

Objects of our investigation were two DNA consequences from complex with 434 Repressor and with purine repressor [2], which represent two types of bending DNA interaction in the minor groove and localized on the same dinucleotide conact (Fig. 1). The results of our simulations make it possible to conclude that there are different levels of relaxation and similar time characteristics of these levels, as well as the different nature of the relaxation dynamics in such complexes.

MATHERIALS AND METHODS

To conduct the experiment two consequences were analyzed. First one d(CAAACTTTCTTG)$_2$ taken from crystal structure of 434 repressor-DNA complex (later as 434 Rep-DNA) [7] and d(GCAAGCGCTTGC)$_2$ from crystal structure of purine repressor-DNA complex (PurR-DNA). Crystal structures were taken from Protein Data Bank (pdb2or1 and pdb1qqa accordingly) [8].

Molecular Dynamics

To perform molecular dynamics, we used a software package NAMD [9] with a force field CHARMM27 [10]. At the preliminary stage preparing a fragment of the DNA molecule

to the molecular dynamics simulation has taken place. Polyionic charge neutralization of DNA was carried out by adding 24 Na^+ counterions at a distance of not less than 5.5 Å from the DNA molecule in a position characterized by the greatest strength of the electrostatic field. Then, a fragment of the DNA molecule was placed in a rectangular water box (model of water molecules TIP3P). The size of the box was determined in the way that DNA molecule is situated at a distance not less than 12 Å in each direction from the borders of the box. In addition, the Na^+ ions and Cl^- were added in concentration corresponding to 0.15 M NaCl to simulate the physiological ionic strength.

Especially for our problem, an algorithm consisting of 2 consecutive phases was developed. In the first phase, which consisted of minimization, heating and equilibration, the atoms of the DNA were fixed. Minimization (1000 steps) and heating to 300 K were performed for 300 ps. Later the system was equilibrated (200 ps) and the second phase (minimization, heating and equilibration) was held with a gradual decrease in the degree of the DNA atoms fixation. In the calculations we used SHAKE algorithm, which allowed using time step of 2 fs. During the minimization and for all subsequent simulations, long-distance electrostatic interactions were calculated by Ewald method (algorithm PME (Particle-Mesh Ewald)) [11]. Constant pressure was maintained by Langevin dynamics (Langevin piston method) [12]. Simulating the dynamics of DNA was carried out in the NPT regime for 2 ns without imposing any restrictions. Structural parameters were calculated using the 3DNA package [13]. Structural parameters were processed using specially written programs in FORTRAN.

RESULTS AND DISCUSSION

Since the bending deformations are described with bending angle (Bend), which characterizes the curve at a particular nucleotide step, we have made simulation time-parameter Bend diagrams. In this paper, our attention was focused on the central dinucleotide contact, where interaction with the protein occurs. Bend angles of adjacent contacts were also investigated, however, the changes in them, were less evident. Angle of bend is calculated as:

$$Bend = \sqrt{Roll^2 + Tilt^2}$$

where the angle of Roll and Tilt angle are the structural parameters of the dinucleotide contact and describe the bending of the duplex inward the groove (Roll) and across the groove (Tilt) [14]. Figure 1 shows the variation of the angle of Bend (Bend) within 2 ns for two systems.

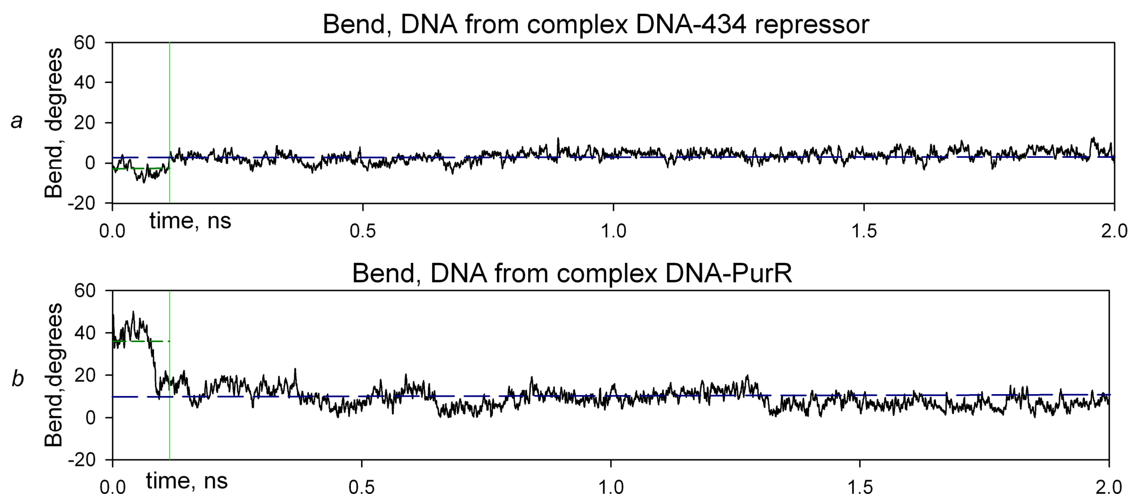

Figure 2. Diagrams describing the change in angle Bend at the central contact TT from a complex of 434 repressor-DNA (*a*) and CG from a complex of purine repressor DNA (*b*) during the simulation of 2 ns. The short dashed line shows the average angle in the first 0,1 ns, long dotted line – mean value for the remaining 1,9 ns.

As it is seen from the diagrams change in bending angle occurs at the same period of time for two systems. The biggest difference is observed within the first 100 ps, when the bending angle takes its maximum value: for a system of 434 Repressor-DNA maximum value of -10.05 (see Fig. 2*a*), for a system of PurR-DNA 50.25 (see Fig. 2*b*) [15]. The negative value of the bending angle for a system of 434 Repressor-DNA is informative and tells about the bending in the opposite direction comparing to system PurR-DNA. Despite the fact that the average bend angle of both systems in the first 100 ps differ by more than 30°, relaxation occurs for the same period of time, but with different relaxation dynamics: for system of PurR-DNA relaxation of the bending deformation was abrupt, for a system of 434 Repressor-DNA – the dynamics of relaxation occurs gradually. Throughout the rest of the simulation values of the bending angle for both systems were slightly changed.

Dynamics of Roll and Tilt change was analyzed as well, as these angles determine the magnitude of the bending angle. These data show that the main contribution to the change of Bend angle and the change in the conformation of DNA in both systems is made by the Roll angle, while the angle Tilt stays almost unchanged (Table 1).

Table 1. Average, minimum and maximum angles Roll, Tilt and Bend in the central contact for a simulation for 2 ns for the first 0,1 ns and further 1,9 ns systems 434 Rep-DNA and PurR-DNA.

	Roll, °				Tilt, °				Bend, °			
	434 Rep-DNA		PurR-DNA		434 Rep-DNA		PurR-DNA		434 Rep-DNA		PurR-DNA	
	0,1ns	1,9ns	0,1ns	1,9ns	0,1ns	1,9ns	0,1ns	1,9ns	0,1ns	1,9ns	0,1ns	1,9ns
Mean	-3,73	3,52	34,98	7,10	4,28	3,24	3,00	-0,4	-3,69	3,53	35,35	9,04
Min	-10,08	-5,64	7,15	-12,19	-0,06	-3,91	-6,9	-13,86	-10,05	-5,63	8,59	0,11
Max	4,29	12,82	50,24	21,91	10,71	9,95	9,78	9,77	4,29	12,82	50,25	23,03

We can assume that the initial 100 ps of DNA interaction with protein in minor groove is more favorable due to the greater availability of DNA atoms. Decrease in the roll angle can be considered as "closure" of dinucleotide contact and for the system 434 Rep-DNA this "closure" occurs gradually, as in the case of PurR-DNA most of the time (80 ps) roll angle retains its value, and "closure" occurs in one moment.

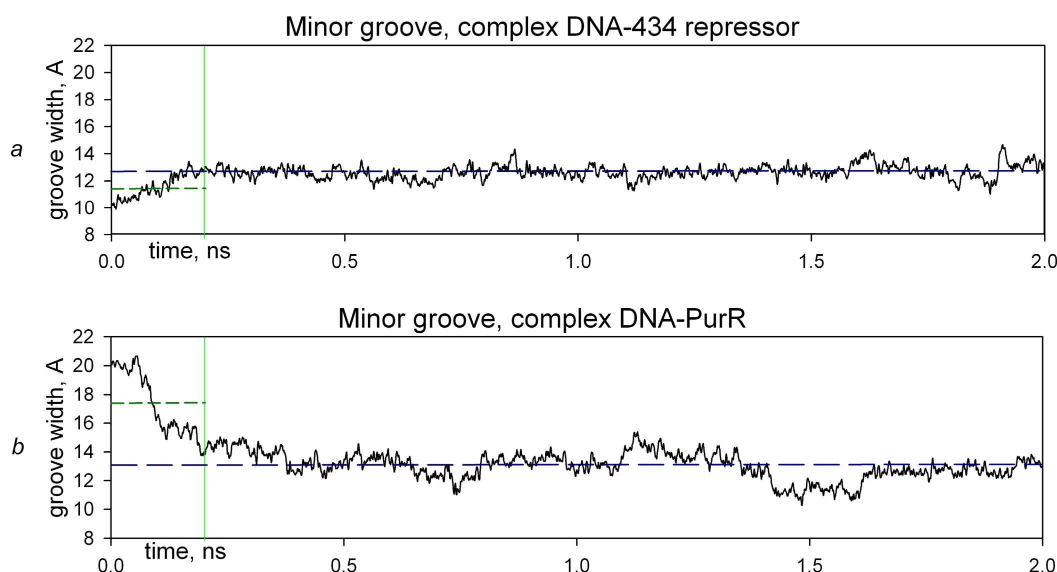

Fig.3 The width of a minor groove in the central contact TT (*a*) and CG (*b*) for 2 ns for complexes of 434 Rep-DNA (*a*) and PurR-DNA (*b*). The short dashed line corresponds to the average width of groove within the first 0,2 ns, long dashed line - the average width of groove during subsequent 1,8 ns.

The next stage of our study was a comparative analysis of the the major and minor grooves width. The width of minor groove of the two systems was differed from the mean value only in the first 200 ps. It should be noted that the width relaxation of the minor groove in the 200 ps was different: for the system 434 Repr-DNA changes of the minor groove width occurred gradually (Fig. 3*a*), whereas for a system of PurR-DNA - abruptly (Fig. 3*b*) [15]. And for PurR-DNA system stepwise dynamics can be divided into two phases, each phase takes about 100 ps.

Throughout the rest of the simulation time (1,8 ns), the width of minor groove remained virtually unchanged and for both systems took approximately the same values (Table 2).

Table 2. Average, minimum and maximum width of minor and major grooves in the central contacts of 434 Rep-DNA and of PurR-DNA for the first 0,2 ns and remaining 1,8 ns.

	Minor Groove width, Å				Major Groove width, Å			
	434 Repr-DNA		PurR-DNA		434 Repr-DNA		PurR-DNA	
	0,2ns	1,8ns	0,2ns	1,8ns	0,2ns	1,8ns	0,2ns	1,8ns
Average	11,59	12,65	17,37	13,04	15,74	15,94	16,35	16,77
Min	9,89	11,03	13,74	10,25	13,06	12,31	14,02	13,4
Max	13,45	14,67	20,67	15,4	18,27	20,81	18,08	20,66

The width of major groove during the entire simulation has not significantly changed and fluctuated in the same range (Fig. 4) for a system with a smooth curved DNA (Fig. 4*a*) as well as for system with a sharp curve of DNA (Fig. 4*b*) [15]. The difference of mean values of major groove width in the initial 200 ps and the subsequent 1800 ps was 0,2 Å (434 Rep-DNA) and 0,42 Å (PurR-DNA), that is not statistically significant counting observed values.

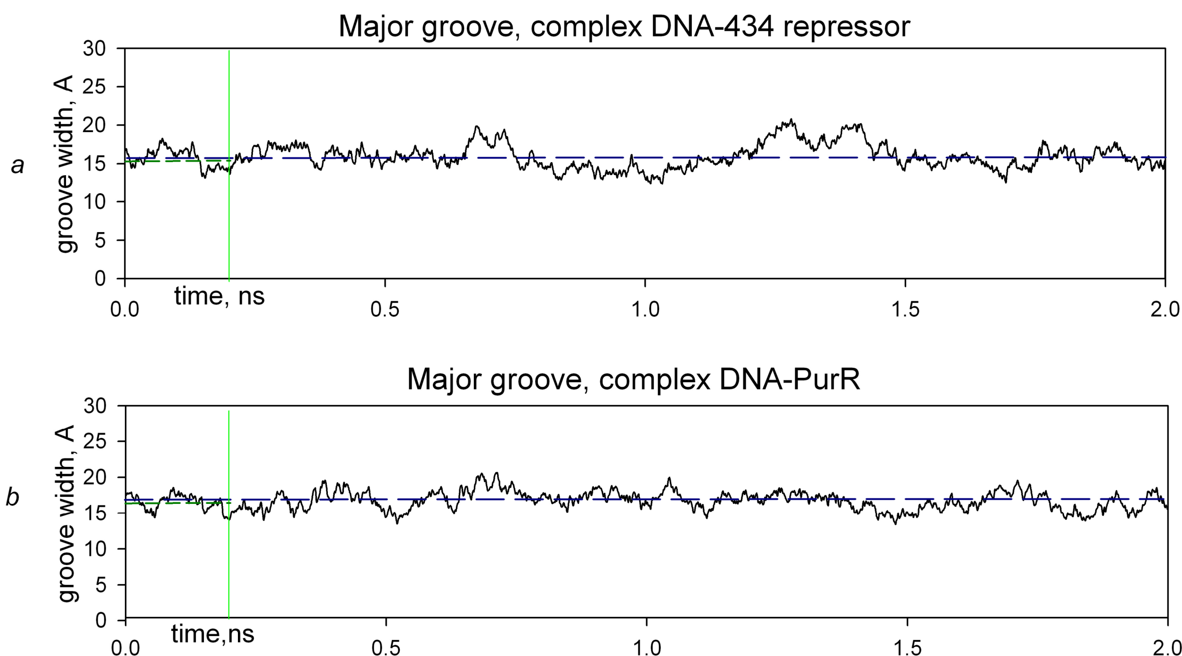

Figure 4. Changes in the values of the major groove width in the central contact TT for the system 434 Rep-DNA (*a*) and CG for system PurR-DNA (*b*) during the 2 ns simulation. The short dashed line corresponds to the average width of groove within the first 0,2 ns, long dashed line - the average width of groove during subsequent 1,8 ns.

So far it can be suggested that interaction with a protein in the minor groove leads to a significant change only of a minor groove width and almost has no effect on the change of a major groove width.

Thus, the results of our simulations indicate the presence of different levels of time relaxation: the level of local structural parameters describing dinucleotide contact (Roll, Bend), the level of the grooves width, which is formed with six base pairs.

CONCLUSIONS

Relaxation mechanisms of two types of DNA bending deformations are similar and can be characterized by the same time characteristics.

A distinctive feature is in the character of relaxation: in the case of smooth curve change from the parameters describing tense DNA conformation to the parameters characterizing relaxed DNA structure, occurs gradually, and in the case of sharp localized curve bending parameters change abruptly. Relaxation of local structural parameters takes less time comparing to the parameters describing duplex as a whole, and that indicates different time levels of relaxation.

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