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ARTICLE

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Motion of groups of atoms in DNA studied by molecular dynamics simulation

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Abstract The analysis of Molecular Dynamics simulations of two double stranded oligonucleotides is presented in terms of motions of quasi rigid subunits. First, a strategy is presented for grouping atoms submitted to concerted internal motions. The method is based on the analysis of the interatomic distance RMS matrix. It is found that each nucleotide can reasonably be decomposed into 3 or 4 rigid groups of atoms depending on the tolerance of the definition of a rigid body. In the second part, the different kinds of motions of the subunits (deformation, translation and rotation) are studied in terms of correlation using the canonical correlation analysis of data. It is shown that the residual deformation of any subunit does not influence the translational and rotational motions of the others, except perhaps for long time dynamics.

Key words Molecular dynamics · DNA · Canonical correlation · Rigid body

Introduction

Molecular Dynamics simulation (MD) allows the study of the deformation of macromolecules at atomic resolution. It consists of computing all the atomic coordinates at different instants separated by a time increment dt, given a model of interatomic interactions (force field). However, owing to the large number of atoms involved in biological systems, and despite the increasing power of computers and the development of new algorithms, only those internal motions which occur on the time range of a few nanoseconds can be investigated. For many purposes this time is not sufficient. This is the case, for example, for monitoring large amplitude deformations, or for comparing the results of a simulation to results from experiments which are sensitive to much longer time scales. Therefore it is necessary to develop other approaches for increasing the time range of simulations. One possibility could be to group atoms within subunits which could be considered as almost rigid and to simulate the dynamics of these rigid bodies instead of simulating the dynamics of all the individual atoms. The dynamics of each subunit would be described with six coordinates (3 for translation and 3 for rotation) instead of the 3 Cartesian coordinates of each atom for the all-atom simulation. Therefore, it is clear that the number of degrees of freedom could be considerably reduced and one expects that, at each step of the simulation, the computation time would be shorter in the case of the rigid body dynamics simulation. Furthermore it is also expected that the overall motions of groups of atoms will be slower than motions of individual atoms, so that the time increment used in the numerical integration of the equations of motion could be larger than that commonly used for the all-atom simulation. It is obvious that the larger the subunits the more important would be the time saving.

However, before trying to perform any rigid body dynamics simulation, two problems have to be solved. The first one concerns the question of which atoms can be grouped in order to form these quasi-rigid subunits. The second comes from the fact that no subunit can actually be perfectly rigid at usual temperatures, and one has to be careful that neglecting the deformation, even if it is small, does not bias the results of the simulation. The present paper deals with these two points and shows how useful information can be obtained from the analysis of a usual all-atom molecular dynamics simulation performed over a limited time range. This information could be subsequently used in a long time rigid body dynamics simulation, but this is not the aim of the present paper.

The following section presents the molecules which have been studied with a few words on the simulation protocol, and describes the methods which were used for defining the quasi-rigid subunits and for testing the influence of the subunit deformations on the full internal dynamics

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of the molecules. The next section presents and discusses the quasi-rigid subunits which were found and correlation studies are presented. Finally a short conclusion is given.

Methods

1 MD simulation

The results which are presented concern double stranded DNA fluctuating around the B conformation. They have been obtained from the analysis of two all-atom MD simulations of two self-complementary oligonucleotides having very different base compositions: the octanucleotide d(CTGATCAG)2 and the dodecanucleotide d(CGCAAATTTGCG)2. The period of analysis covers 200 ps and 250 ps for the 8-mer and the 12-mer respectively. Both molecules were simulated with the GROMOS 87 program (van Gunsteren and Berendsen 1987) in the presence of explicit water molecules (1451 for the 8-mer, 2500 for the 12-mer) and Na^+ ions for balancing the fully charged phosphate groups (14 for the 8-mer, 22 for the 12-mer). Special attention was paid to the thermal equilibration of the systems. The detailed description of the simulation protocol may be found in Briki and Genest (1993, 1995) for the octanucleotide and in Gaudin et al. (1997 a) for the dodecanucleotide.

The quality of the dodecanucleotide simulation was checked by comparison with NMR experiments using different criteria (Gaudin et al. 1997 a): i) The NMR structure taken as initial structure for the dynamics, was conserved during the simulation; ii) NOE build-up curves between different pairs of protons (aromatic with H1′, H2′ and H2″, either intra- or inter-nucleotide) were calculated from the trajectory and were found to fit the experimental curves satisfactorily, iii) the internal correlation times and order parameters of the Lipari-Szabo theory (Lipari and Szabo 1982) describing C1′ relaxation were also calculated from MD trajectories and were in good agreement with those determined by ^{13}C NMR experiments.

2 Search for quasi-rigid subunits

This search is based on the analysis of the root mean square fluctuations of the interatomic distances (RMS). For a molecule with N atoms an N*N matrix is built which contains the RMS's of all the atom pairs:

$$RMS\,(i,j) = \{(1/K)\,\Sigma\,[d_{ij}\,(k) - \langle d_{ij}\rangle]^2 >\}^{1/2} \qquad (1)$$

In this expression, K is the number of configurations stored during the MD simulation, $d_{ij}(k)$ is the distance betweeen atoms i and j in the k^{th} configuration and $\langle d_{ij}\rangle$ is the average value of this distance. The summation in Eq. (1) is over all the conformations.

By definition, a rigid body is composed of points whose mutual distances are constant. Thus, searching for quasi-rigid subunits consists of extracting square sub-matrices from RMS(i, j) in which all the elements are sufficiently small. Different methods have been used. In some of them a tolerance r_c for the definition of a rigid body is chosen a priori. This means that a set of atoms is considered as belonging to the same rigid subunit if the mean square fluctuation of each of their mutual distances is smaller than r_c. A graphic representation of the RMS matrix can therefore be given in which each element smaller than r_c is represented as an elementary black square and each element greater than r_c is represented by a white square. If a set of atoms belongs to the same rigid subunit, it will appear as a large black square on the map, if the atoms are labelled consecutively. Therefore, by moving rows and columns of the RMS matrix it is possible to sort, either manually or automatically, sets of atoms which can be grouped together according to the tolerance criterion. At this point we must mention that there is no unique solution for partitioning the atoms, and no objective criteria can be used for selecting a particular solution. Finding the whole set of solutions is very time consuming (Nichols et al. 1995), and ultimately of little practical interest. We have preferred to determine a single solution in a two step process.

First, a fast automatic method described in Gaudin et al. (1997 b) proposes an arbitrary solution, which depends on the numbering of the atoms of the molecule. The total number of subunits is relatively insensitive to this numbering, depending mostly on r_c.

In the second step, a fast method called dynamical clustering is used, which does not require an explicit choice of the tolerance r_c, but the total number of subunits to be sorted needs to be defined (Gaudin et al. 1997 b). This number is obtained from the first method.

It has been shown that using such a strategy leads to reproducible and reasonable results. Of course, the results of this analysis depend on the tolerance for the definition of the rigid subunits.

3 Canonical correlation analysis

The full internal dynamics of a subunit can be decomposed into three components which are the overall translation, the overall rotation and the deformation. Each of these motions is described by a different set of coordinates. For the full dynamics and the deformation they are the Cartesian coordinates of all the subunit atoms, defined in a frame bound to the molecule and to the subunit respectively. The translation and the rotation are described by the three coordinates of the center of mass and three angular coordinates (Euler angles or quaternions) respectively.

If one considers two subunits, it is of interest to determine how the different kinds of motion of the first one are correlated to the motion of the second. Mathematically one has to quantify the global correlation between two sets of variables by a single coefficient. This is not a trivial problem. We have used a method known as canonical correlation analysis of data (Saporta 1990). It has been described recently (Briki and Genest 1994, 1995; Genest 1996; Hery et al. 1997), and the method will be briefly outlined here.

Let A and B be two sets of random variables $\{a_i\}$ and $\{b_i\}$ containing n and m elements respectively (we will asume $n \leq m$). We assume the distributions of the a_i's and b_i's to be centered and normalized. Suppose that each of the variables a_i or b_i is sampled by K values $a_i(k)$ or $b_i(k)$ ($k = 1 \ldots K$, $K \geq m$) which can be considered as the components of a K-dimensional vector $\mathbf{a}_i$ or $\mathbf{b}_i$. This means that each vector $\mathbf{a}_i$ or $\mathbf{b}_i$ is an element of the K-dimensional space R^K. Thus A and B define n-dimensional and m-dimensional subspaces of R^K, E_A and E_B respectively. The definition of a global correlation coefficient between A and B is related to the relative positions in R^K of E_A and E_B. Let us define two sets of orthogonal normalized vectors $\{\mathbf{e}_i^A\}$ and $\{\mathbf{e}_i^B\}$ in E_A and E_B respectively obeying the following criteria: each vector $\mathbf{e}_i^A$ (or $\mathbf{e}_i^B$) corresponds to at most one vector $\mathbf{e}_i^B$ (or $\mathbf{e}_i^A$) which is not orthogonal to it. The vectors $\{\mathbf{e}_i^A\}$ and $\{\mathbf{e}_i^B\}$ are called the canonical vectors of E_A and E_B respectively, and the dot product $\mathbf{e}_i^A \cdot \mathbf{e}_i^B$ is called the canonical correlation coeffient between $\mathbf{e}_i^A$ and $\mathbf{e}_i^B$. It represents the cosine of the angle formed by both vectors. If the $\mathbf{a}_i$'s (and $\mathbf{b}_i$'s) are linearly independent, there are n vectors $\mathbf{e}_i^A$ (and m vectors $\mathbf{e}_i^B$) and there are at most n canonical correlation coefficients different from 0. A global canonical coefficient between A and B may be defined as the quadratic average of the different canonical coefficients. With this definition it is not necessary to explicitly calculate the canonical vectors, and we can proceed as follows:

– construct the following usual correlation matrices:

$$R_{AA}(i, j) = \langle a_i\, a_j \rangle \qquad (i, j = 1 \ldots n) \qquad (2\,a)$$

$$R_{BB}(i, j) = \langle b_i\, b_j \rangle \qquad (i, j = 1 \ldots m) \qquad (2\,b)$$

$$R_{AB}(i, j) = \langle a_i\, b_j \rangle \qquad (i = 1 \ldots n,\ j = 1 \ldots m) \qquad (2\,c)$$

$$R_{BA}(i, j) = \langle b_i\, a_j \rangle \qquad (i = 1 \ldots m,\ j = 1 \ldots n) \qquad (2\,d)$$

– invert R_{AA} and R_{BB}

– calculate the following product:

$$R = R_{AA}^{-1} \cdot R_{AB} \cdot R_{BB}^{-1} \cdot R_{BA} \qquad (3)$$

R is then the canonical correlation matrix. It is a square matrix and it can be demonstrated that its eigenvalues are the squares of the canonical correlation coefficients (Saporta 1990), so that the global canonical correlation coefficient M between both sets A and B is obtained from the trace of R as:

$$M = \{(1/n)\ \mathrm{Trace}\ [R]\}^{1/2} \qquad (4)$$

M is a scalar comprised between 0 and 1. $M = 0$ means that A and B are completely independent (E_A and E_B are orthogonal), while $M = 1$ means that both groups describe the same properties (E_A is included in E_B).

For our purpose, A and B correspond to the kinds of motion, the variables $\{a_i\}$ and $\{b_i\}$ to the normalized fluctuations of the corresponding coordinates and K to the number of configurations stored during the simulation. If the averages in Eq. (2) are calculated with the values of the variables of A and B taken at the same time one gets an equal time canonical correlation coefficient. If they are computed using a time delay between the variables of B and the variables of A, and if the delay is allowed to vary one gets a time-dependent canonical correlation function, either an autocorrelation function, if A and B contain the same variables, or a cross-correlation function, if A and B contain different variables.

Results and discussion

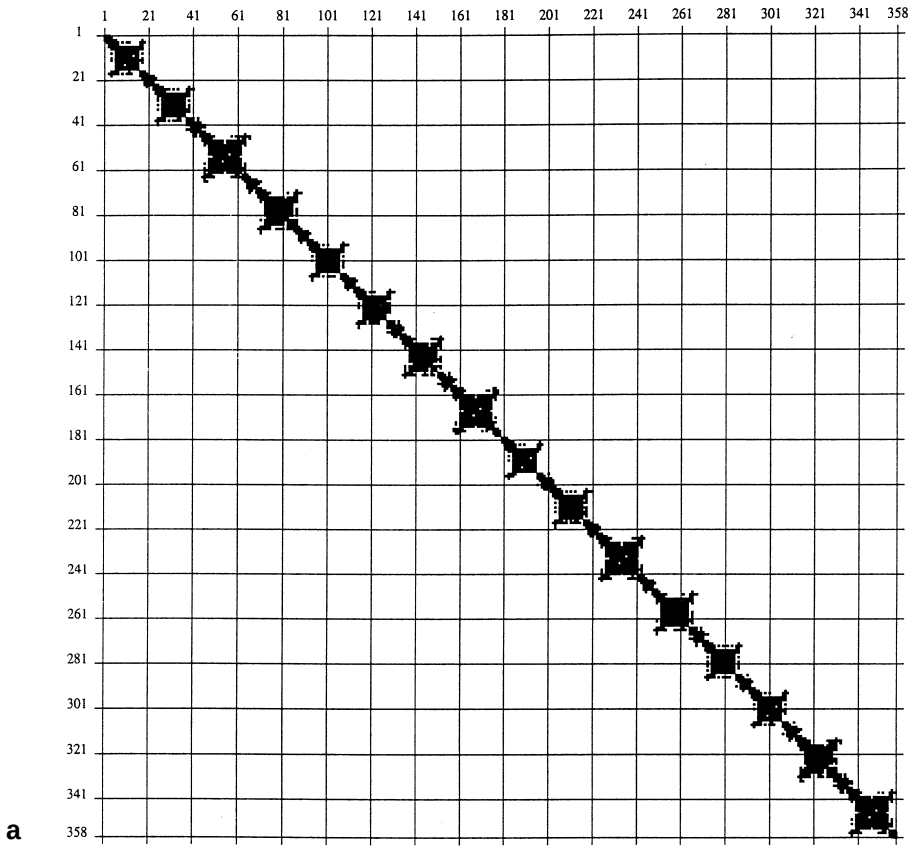
1 Determination of the quasi rigid subunits

A set of RMS matrices corresponding to a tolerance r_c ranging between 0.007 nm and 0.1 nm were calculated. As an example, Fig. 1 shows the graphic representations of RMS matrices corresponding to the 8-mer, obtained for three different values of r_c (0.012, 0.028 and 0.050 nm). For these values, the first method for partitioning the atoms detects roughly 60, 45 and 32 groups respectively. This corresponds approximatively to 4, 3 and 2 quasi-rigid subunits per nucleotide on average. For the 12-mer, the same values are obtained for the same tolerances. Of course the composition of the groups sorted with this method is very dependent on the labelling of the atoms (see Method section). However a detailed examination of RMS (i, j) and the application of the second method of partitioning shows that for $r_c = 0.028$ nm, each nucleotide can be described by 3 rigid subunits which are the base, the sugar ring and the backbone atoms PO_4 + C5′ respectively. These 3 rigid (mechanical) entities overlap the three chemical entities comprising the nucleic acids. However, we note that the C5′ may often be sorted with the atoms of the sugar ring, but not for all residues.

For $r_c = 0.012$ nm the main difference is that the C5′ cannot be regrouped with all the atoms of the PO_4 group and must be considered as a subunit by itself. Therefore with this tolerance which corresponds to roughly 2.5 times the bond length fluctuations at room temperature, each nucleotide can be thought of as four rigid subunits, one of them containing only one atom. More details of this analysis can be found in Gaudin et al. (1997 b).

For higher values of r_c it was not possible to establish a systematic relationship between the quasi-rigid subunits and specific groups of atoms in each nucleotide. It can be seen in Fig. 1 that, as r_c increases, some atoms belonging to paired bases seem to have concerted motions, but the notion of a rigid subunit is questionable. In the following the rigid subunits will be considered on the basis of a tolerance of 0.028 nm.

It has been previously shown (Briki and Genest 1995) that the fluctuations of the position of any given atom are much smaller (by more than an order of magnitude) if the position is defined in a frame bound to the subunit to which it belongs rather than to a frame bound to the molecule. This validates the description of nucleotides as a few quasi-rigid groups of atoms. It was also found (Gaudin et al. 1997 b) that the difference between the electrostatic potentials generated by two random instantaneous conformations of a subunit and computed with a given force field,



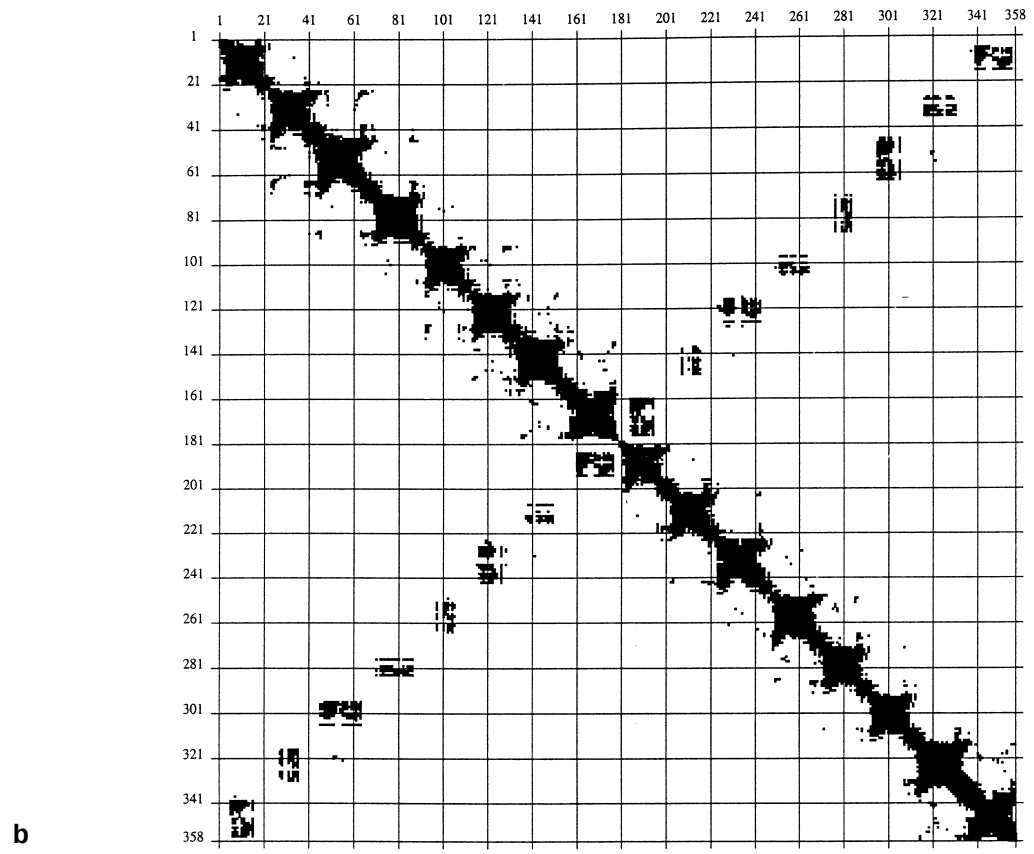
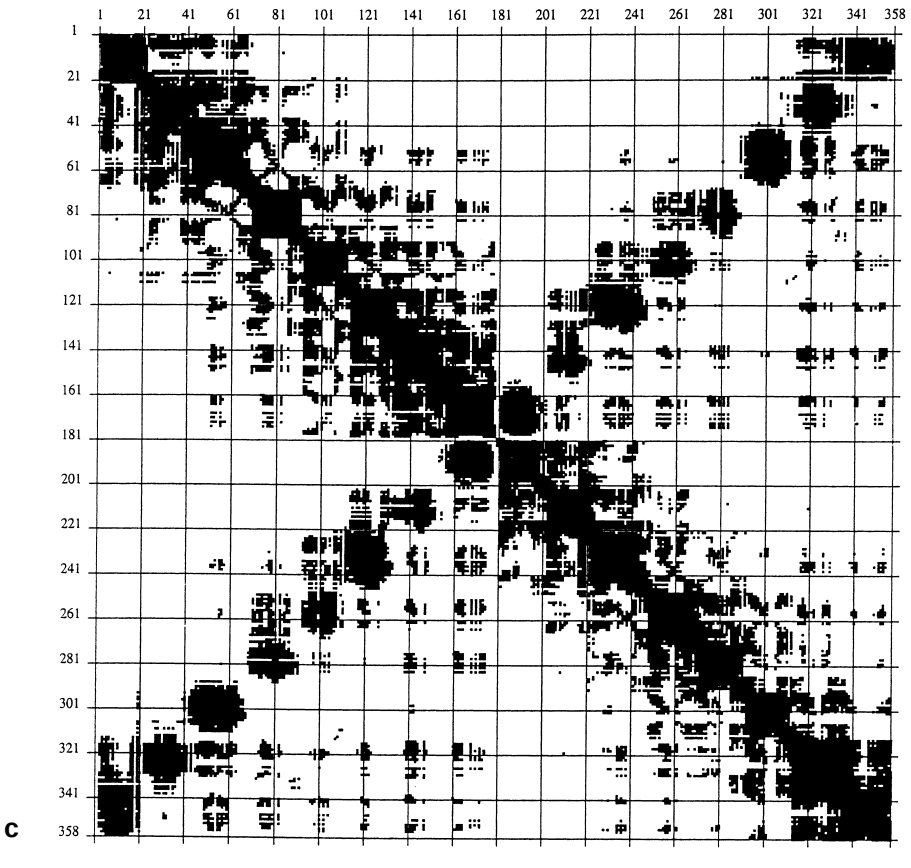


Fig. 1 a–c Graphic representation of the interatomic distance RMS matrix of the 8-mer obtained with different values of r_c: 0.012 nm (**a**), 0.028 nm (**b**) and 0.050 nm (**c**)



c

is not greater than the difference between the potentials of a single conformation calculated with two different force fields (CHARMM and GROMOS). This is true for all the sugar rings and the bases, but for the atoms of the backbone the C5′ must be excluded.

Table 1 Equal time canonical correlation coefficients between the different kinds of motion of the backbone group of atoms of residue G3 of the 8-mer and the full dynamics of the sugar ring groups of atoms of the same residue (a) and of residue A7 (b)

Translation	Rotation	Deformation	Full dynamics
0.89 (a)	0.68 (a)	0.12 (a)	0.58 (a)
0.49 (b)	0.37 (b)	0.18 (b)	0.31 (b)

2 Correlation study

Equal time correlation

Having defined quasi-rigid subunits in the previous section, it is now important to check how their small deformations may influence the full dynamics of another subunit. This has been done for the octanucleotide. As an example, Table 1 gives the equal time canonical correlation coefficients between the three different kinds of motion of the set of backbone atoms (PO_4 + C5′) of G3 and the full dynamics of the covalently linked sugar ring of the same residue. The backbone group including the C5′ is chosen because it corresponds to the worst case, its internal fluctuations are higher than for either the bases or the sugar rings. It is obvious that the position of the center of mass and the orientation of the backbone atoms have a strong influence on the dynamics of the sugar ring. The influence is actually more important for the position than for the orientation. By contrast, the very small correlation between the deformation of the backbone and the dynamics of the sugar ring shows that the conformation of the backbone does not significantly influence the full dynamics of the ring. The same behaviour is observed between any two covalently linked subunits (Briki and Genest 1995).

An interesting point still to be investigated concerns how far along the nucleic acid sequence the different kinds of motion of a subunit influence the global dynamics of another subunit. Table 1 gives, as an example, the canonical correlation coefficients between the motions of the backbone subunit of G3 (including the C5′) and the full dynamics of the sugar ring subunit of A7. These subunits are separated by 11 covalent bonds. The deformation of the G3 backbone subunit shows no influence on the dynamical behaviour of the A7 sugar ring. The influence of the translation and rotation is not negligible, although it is less important than for directly linked subunits. A detailed analysis for different sets of subunits may be found in Briki and Genest (1995).

According to this equal time correlation study, it thus appears that considering the subunits defined in the previ-

ous section as true rigid bodies is an appropriate assumption for describing the internal dynamics of these double stranded B-DNA oligonucleotides.

Time dependent correlation functions

Autocorrelation. Canonical autocorrelation functions for the overall translation and rotation of 12 different subunits belonging to the two most central base pairs of the octanucleotide were calculated in the range 0–20 ps corresponding to a tenth of the production period of the simulation used for averaging in Eq. (2). Calculating the correlation functions over a larger interval would result in a loss of accuracy for times greater than 20 ps. All the normalized autocorrelation functions can be fitted with a biexponential decay:

$$C(t) = a \exp(-t/T1) + (1-a) \exp(-t/T2)$$

Table 2 gives the average values of the different parameters obtained for translational and rotational motions. Very fast motions on the time scale of a few tenths of ps are responsible for a fast, but limited, loss of memory of the initial positions of the center of mass and the initial orientation of the subunits. A slower decorrelation is observed in the range 10–20 ps. For all the subunits that have been examined the loss of memory was always faster for rotation than for translation. A mean correlation time for both kinds of motion, defined as $\langle T \rangle = \langle a\ T1 \rangle + \langle (1\text{-}a)\ T2 \rangle$, is also given in Table 2. The value of the long rotational correlation times T2 (and also of $\langle T \rangle$) are very similar to the rotational correlation times of the C1′–H1′ sugar bond which have been experimentally determined by NMR (Gaudin et al. 1995) as can be seen in Table 2.

The normalized canonical autocorrelation functions for the deformation of the same central subunits were also calculated, but the corresponding correlation times could not be determined owing to the very fast decrease from 1 to a plateau value around 0.2 in less than 0.3 ps, corresponding to complete decorrelation. Therefore each subunit very rapidly loses the memory of its conformation.

At this point it is established that the conformation of a subunit at a given time has no effect on the position and the orientation of the other subunits at the same time (previous section) and no effect on the conformation of the same subunit after a delay of a few tenths of picoseconds (this section).

Table 2 Mean parameters for a biexponential fitting of translational and rotational normalized autocorrelation functions of the 8-mer. T1 and T2 are the correlation times, a the proportion of the fast components, $\langle T \rangle$ the weighted mean correlation time and T exp the mean experimental correlation time of C1′ H1′ bonds determined by ^{13}C NMR in Gaudin et al. (1995). a, T1 and T2 correspond to an average over all the subunits of the two central base pairs. The maximum deviation is given in brackets. Time is in ps

	a	T1	T2	$\langle T \rangle$	T exp
Translation	0.35 (0.1)	1.0 (0.5)	19. (3.)	12.7 (2.5)	–
Rotation	0.35 (0.1)	0.15 (0.05)	11. (2.)	7.2 (2.)	10.

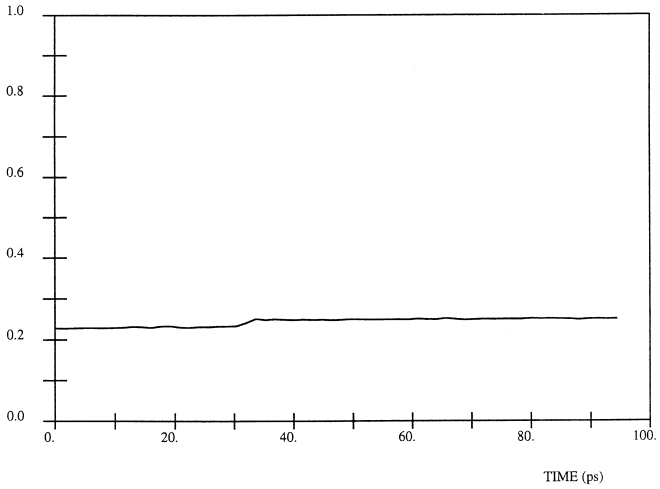


Fig. 2 Cross-correlation function between the deformations of backbone atoms of residue A4 and sugar ring atoms of residue T5 of the 8-mer

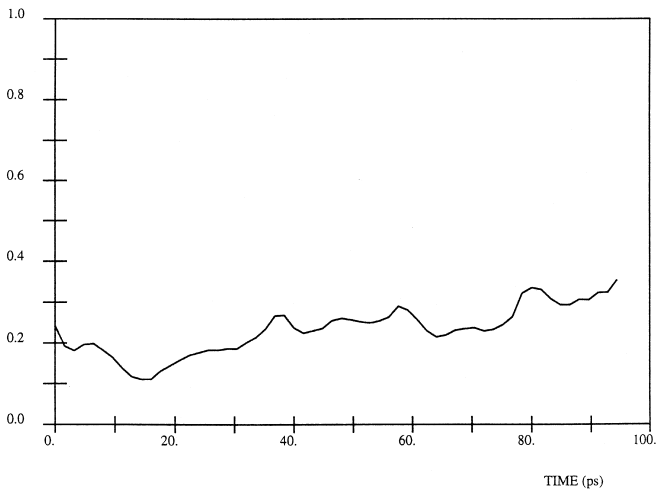


Fig. 3 Cross-correlation function between the deformation of sugar ring atoms of residue A4 of the 8-mer and the translation of the base atoms of the same residue

Cross-correlation. We examine here how the conformation of a subunit at a given time influences the conformation, orientation and position of the other subunits after a time delay. Cross correlation functions between deformations of different subunits remain constant with a low value at all times. This is illustrated in Fig. 2, and holds even if the correlation functions are computed over a time range of 95 ps. Thus the conformation of any subunit at any given time has no influence on the conformation of the other subunits even after a delay of almost 100 ps.

Finally, cross correlation functions between the deformation of a subunit and the rotational and translational motions of other subunits were evaluated. In both cases the

correlation is very weak (less than 0.25) in the first 20 ps. However, despite a loss of accuracy in the last part of the functions, a slow, but systematic, increase of the correlation functions is observed as time increases. An example is given in Fig. 3. Starting from a very weak correlation at time 0 ps, moderate correlation values of 0.3 – 0.35 are obtained after 95 ps. This could indicate that the conformation of any subunit at a given time may slightly influence the long time dynamics of the molecule. But at the present time it is not clear whether the delayed correlation observed in the range 20 – 100 ps is significant or if it is due to statistical artefacts. This point will be elucidated by analysing longer trajectories covering roughly 1 nanosecond. Correlation functions will then have a better accuracy in the range 20 – 100 ps than in the present work.

Examination of Fig. 2 needs some comments. It appears that the correlation function remains at a remarkably fixed value of about 0.2. One should rather expect a correlation fluctuating around zero at long times, and one may think that there is a base line problem. In fact, the observed nonzero value is the consequence of the definition of the correlation function which is calculated as a positive root square, i. e. the absolute value of the real correlation. Consequently, the calculated value fluctuates between positive limits, leading to a systematic overestimation in the case of weak correlation values. This artefact is not expected for higher values of the absolute correlation, because the fluctuations around the average would not change the sign of the real correlation.

Conclusion

This study has shown that, to a good approximation, the internal dynamics of the B-DNA molecule can be described by the concerted motions of groups of atoms moving as rigid bodies. Methods for defining the rigid bodies from a molecular dynamics simulation are presented. It is found that, according to the tolerance used for defining a rigid body, each nucleotide may be decomposed into three or four quasi-rigid subunits. It is shown that the small deformations of these subunits do not affect the overall dynamics, except for a possible weak influence on the long time behaviour. Fourier transforms of the trajectories of a few translational and rotational components suggest that the time increment could be of the order of 10^{-14} sec if rigid body dynamics simulations were performed with the subunits described in this study.

It is important to realize that any deformation of a group of atoms modifies the mass distribution and the tensor of inertia of the group. Therefore, the fitting process has two major consequences which actually describe the deformation i) displacement of the center of mass and ii) reorientation of the principal axes. These two points appear to be of major importance for the dynamical behaviour of the molecules studied in the present work.

All the results reported here concern a time scale of 200 ps and structural fluctuations around the B form of DNA. Larger amplitude motions, such as transitions between canonical forms, may change the present conclusions. However, in the simulations analyzed in the present study, transitions of the sugar puckering between north and south conformations sometimes occur, although they are too rare to be accurately sampled. They are a possible cause for the weak correlation observed at long time between the deformation of a subunit and the rigid body motions of other subunits. Nevertheless, as stated above, the main consequences of this transition can certainly be analyzed as a translation and a rotation of the furanose ring.

It is finally remarked that the methods described in the present paper may be equally used for analysing the MD trajectories of proteins (Hery et al. 1997).

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