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Comparison of the solution structures of a DNA dodecamer using NOE and residual dipolar coupling data

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Abstract

The solution structure of a DNA dodecamer is determined by combining dipolar cross-relaxation and residual dipolar coupling (RDCs) data. The ^{13}C–^{1}H RDCs result from the partial alignment of the molecule after its dissolution in different oriented dilute liquid crystals: polyethylene glycols, bicelles and phages. It clearly appears that thanks to the use of RDCs, the global curvature of the duplex solution structures is reduced. This is shown to result from the use of long-range RDC restraints and from the symmetry properties of a self-complementary dodecamer. ***To cite this article: F. Alvarez-Salgado, et al., C.R. Chimie 7 (2004).***

Résumé

La structure en solution d'un dodécamère d'ADN d(GACTGTACAGTC)$_2$ est déterminée en combinant les informations de vitesses de relaxation croisée et les couplages dipolaires résiduels ^{13}C–^{1}H (RDCs) sont dus à l'alignement partiel de la molécule induit par sa dissolution dans des cristaux liquides dilués orientés : polyethylène glycols, bicelles et phages. Grâce à l'utilisation de RDCs, la courbure globale des structures en solution du dodécamère est réduite. Nous démontrons que cela résulte de la longue portée des contraintes RDCs et des propriétés de symétrie propres à un fragment auto-complémentaire d'ADN. ***Pour citer cet article : F. Alvarez-Salgado, et al., C.R. Chimie 7 (2004).***

Keywords: NMR; DNA; Dilute liquid crystals; Residual dipolar couplings

Mots clés : RMN ; ADN ; Cristaux liquides dilués ; Couplages résiduels dipolaires

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1. Introduction

The cell genetic information is carried by DNA. Naturally, the DNA double helix can be modified by different endogenous mechanisms, such as spontaneous deamination, alkylation, mismatch formation or damage induced by oxygen radical attack [1]. Any chemical alteration of the DNA molecule may lead to dramatic consequences on the encoded proteins and then on the cell viability [2]. In order to preserve the DNA integrity, different enzymatic systems present in the cell continuously check and repair it. One of the assumptions to explain how lesions are detected among the billions base pairs is that they induce a local curvature of the DNA structure. The aim of the present study is to validate a robust procedure to determine the overall shape of DNA fragments in solution. In particular, we study a self-complementary dodecamer $d(GACTGTACAGTC)_2$ by combining standard NMR experimental data, proton–proton distances derived from NOE [3], with long-range restraints. The latter are obtained via the measurement of one-bond ^{13}C–^{1}H residual dipolar couplings (RDC), enhanced by the dissolution of the biomolecule in dilute liquid crystals, in order to provide orientational restraints [4].

2. Materials and methods

2.1. NMR measurements

After freeze-drying, the non-labelled DNA dodecamer, provided by Genset, was dissolved in a D_2O solution containing 10 mM sodium phosphate, 0.2 mM ethylenedinitrotetraacetic acid (EDTA), 50 mM NaCl. The final concentration of the duplex was 0.5 mM and the pH was 7. All NMR experiments were carried out on a Bruker DRX-600 spectrometer equipped with a ^{15}N–broadband–^{1}H inverse probehead and on a DRX-800 spectometer equipped either with a ^{13}C–^{15}N–^{1}H inverse classical probehead or a ^{13}C–^{15}N–^{1}H inverse cryo-probehead. The peaks were assigned through classical ^{1}H NMR procedures based on DQF–COSY and NOESY spectra. The proton distance constraints were derived from NOESY build-up curves composed of eight mixing times (ranging from 30 to 300 ms) acquired at 600 MHz. For the measurement of RDCs, three different media were used: (i) negatively charged bicelles composed of dimyristoyl-phosphatidylcholine (DMPC), dihexanoylphosphatidylcholine (DHPC) and sodium dodecyl sulfate (SDS) (30:10:1) at a concentration of 5% w/v [5], (ii) filamentous phages pf1 at a concentration of 20 mg ml^{-1} [6] provided by ASLA and (iii) 5–alkyl–poly(ethylene glycol)/5-alkyl alcohol (PEG) at a concentration of 5% w/v [7]. The extraction of RDCs was obtained by comparing the anti-phase ^{13}C–^{1}H splittings in the direct dimension of non-decoupled 2D HSQC spectra acquired in oriented and isotropic solutions. The measurements of the one-bond $^{1}J_{CH}+^{1}D_{CH}$ couplings were carried out by peak-picking of the F2 slices using the Bruker Xwinnmr software and the Decord software [8].

2.2. Structure calculations

The molecular dynamic simulations were performed with CNS 1.1 software [9] running under Linux RedHat 7.1 using two types of restraints: experimental restraints (204 NOE and 3 sets of 48 $^{1}D_{CH}$ each corresponding to the 24 $C_{1'}$–$H_{1'}$ and to the 24 $C_{6,8}$–$H_{6,8}$) and standard restraints (based on canonical structures: planarity bases, torsion angles and hydrogen-bond distances). Structures were computed in two steps both using simulated annealing. (*i*) In the first step, starting from an extended structure (non-paired strands) and random velocities, a 60 ps high-temperature molecular-dynamics simulation (MD) in torsional angle mode [10] at 20 000 K, with all constraints excepted the RDCs, was applied with a 15 fs time step. A small weight of the van der Waals repulsive term was used at high temperature in order to enable large conformational sampling. During the cooling down procedure, two successive modes were used: firstly a torsional angle dynamics from 20 000 to 1000 K, then a cartesian dynamics from 1000 to 300 K. A Powell energy minimization was then performed in order to relax the here-after called NOE structures. (*ii*) In the second step, for each set of RDCs, corresponding to one out of the three media, axial and rhombic components of the dipolar alignment tensor were computed using the average NOE structure and the experimental RDC restraints, thanks to a home-written C program [8]. The structures were then refined by adding the RDC restraints to the previous restraints. The following protocol was used: heating at 1000 K, MD in torsional angle mode from 1000 to 300 K, followed by

Table 1
Average deviations for the accepted structures among the 100 calculated ones compared to the idealized covalent geometry for the different restraints used in the simulated annealing procedure. The last line corresponds to the average curvature for the accepted structures

	NOE alone	pf1	PEG	Bicelles
NOE restraints (Å)	0.04	0.03	0.03	0.03
Dihedral angle restraints (deg)	0.44	5.89	5.85	7.77
Improper torsions (deg)	0.32	0.84	0.75	0.86
Global Axis Curvature (deg)	20.5 ± 2.6	6.2 ± 3.7	12 ± 5.2	10.2 ± 7.7

a MD in cartesian mode at 300 K and finally a Powell minimization. Three families of structures corresponding to the three media used to enhance the RDC: bicelles, PEG and pf1, were thus obtained. The DNA structural properties were analysed using the CURVES 5.1 algorithm [11].

3. Results

RDCs $^{1}D_{CH}$ are extracted from non decoupled HSQC spectra. Typical subspectra are represented in Fig. 1. For each of three media $^{1}D_{CH}$ values ranging between –14 Hz and 15 Hz are obtained with an estimated uncertainty of 1 to 2 Hz. In parallel, 204 proton–proton distance restraints are derived from NOE build-up curves.

Whatever the type of constraints used to determine the solution structure of the DNA duplex (with or without RDC), the calculations finally converge to a B-DNA type structure. Globally, all bases are in the *anti* conformation and the osidic units are predominantly in a C2′-*endo* conformation, in agreement with the vicinal scalar couplings measured on the DQF–COSY spectra. The main difference between the families of structures lies in the global shape of the DNA (Table 1).

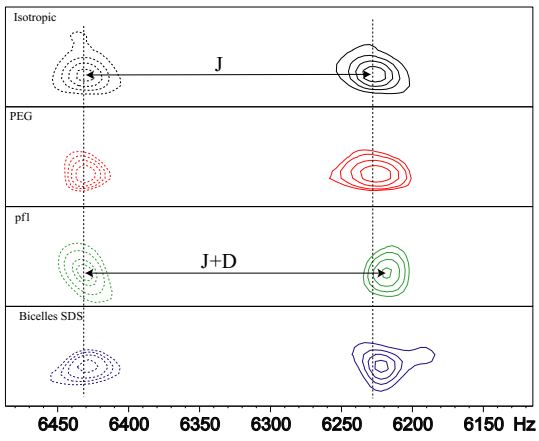

Fig. 1. 2D HSQC sub-spectra of the DNA duplex in four different media. The cross-peaks are those of adenine-2 C_2H_2.

When the NOE restraints are used to determine the solution structures of the duplex, the average structure (Fig. 2) exhibits a non-negligible (~ 20°) global axis curvature as computed using Curves software (Table 1, column 1); similar curvatures are obtained using Madbend [12]. We have however checked that the NOE restraints are compatible with a canonical B-DNA structure with no curvature. This proves that these local restraints are in fact compatible with DNA structures exhibiting a large range of curvature, and substantiates

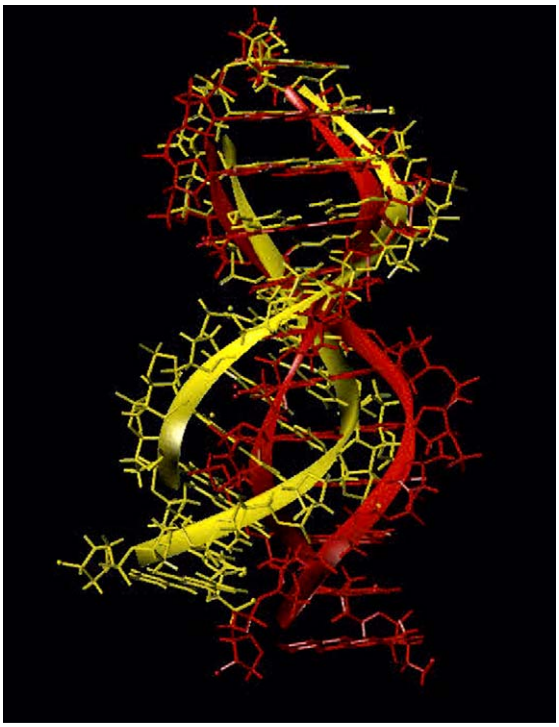

Fig. 2. Superposition of two average DNA structures. The structure alignment is based on a match of the guanine G1. In red the structure represents the average of the structures obtained with the RDC measured in pf1 media, in yellow the average of the NOE structures.

the need for long-range restraints for correctly defining the overall DNA shape [13–15]. Each set of RDCs leads to a different average structure. All accepted structures have similar covalent, electrostatic, van der Waals and NOE energy values. They are close to the energies of the NOE structures, but larger deviations from idealized covalent geometry are encountered for the dihedral and improper torsion angles (Table 1). The global axis curvature for the accepted structures obtained with the RDC restraints is smaller (between 6 and 12°) than for the NOE structures. Even if the three alignment tensor directions corresponding to the three sets of RDCs are not very different, we were nevertheless unable to obtain acceptable structures with the simultaneous use of the NOE and of the three sets of RDCs restraints. This lack of convergence appears either as RDC violations when small RDC force constants are used in the simulated annealing procedure or as structural angle distortions when large RDC force constants are used. Different reasons may explain the origin of this behaviour: extracted values of the axial and rhombic components of the dipolar alignment tensors, experimental uncertainties on the RDCs, or small structural change due to the nearby presence of mesogens,...

4. Discussion

The pairwise root mean square deviation (RMSD) (Table 2) of the average structure coordinates shows that the three DNA structures obtained with RDC restraints are similar (RMSD inferior to 1 Å) but differ from the NOE structure (RMSD larger than 1 Å). This is clearly observed on the 3D structures (Fig. 2). The small variations between accepted RDC structures could result either from uncertainties on the RDC or from small structural changes induced by the different mesogens. In all cases, the main result is that the accepted RDC structures exhibit a much smaller global curvature than the NOE structures (Table 1, Fig. 2).

Table 2
Pairwise RMSD in Å between average structures. Only the heavy atoms are considered in the computation.

	PEG	Bicelles	pfl
NOE	1.60	1.24	1.66
PEG		0.75	0.69
Bicelles			0.97

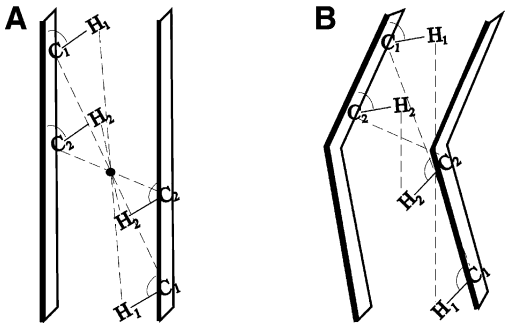

Fig. 3. Schematic representation of straight (**a**) and bent (**b**) DNA structures. The *local* orientation of the C–H spin pairs relative to the DNA strands are identical in the two cases. However, it is only in the case of a straight DNA structure, i.e. when a centre of symmetry is present, that the orientations of the two symmetrical C–H pairs are identical relative to a global molecular tensor frame, leading to identical RDC. In contrast, in the case (**b**), since the C–H exhibit different orientation with respect to the molecular axes, different RDC are expected for the chemically symmetrical C–H pairs. It should be noted that the presence of a straight DNA structure does not prevent the alignment tensor to be fully asymmetric.

Beyond the fact that the duplex sequence is in favour of a straight DNA [16], a physical element substantiates the convergence of the minimization procedure with RDC restraints towards a non-bent structure. Indeed the studied DNA duplex is composed of two self-complementary strands d(GACTGTACAGTC)$_2$. As a consequence, its chemical formula exhibits a centre of symmetry. On the NMR point of view, this property leads usually to the degeneracy of the chemical shift values of two nuclei belonging to nucleotides in the same position of each strand. The centre of symmetry of the structure is however destroyed, if the molecule is bent. This cannot lead to differentiation of the chemical shifts between two strands, since the presence of very-long-range deformations does not largely modify their local magnetic environment. On the contrary, the RDC of two symmetrical pairs of spins are expected to be different (Fig. 3). This would directly affect the non-decoupled HSQC spectra, since additional splitting would appear. A careful analysis of our 2D spectra does not reveal this feature (no additional splitting), demonstrating that the centre of symmetry is preserved at the molecular structure level, and therefore that the DNA structure should not be bent.

5. Conclusions

Based on different numerical or experimental studies [13–17], the canonical structure in solution of small DNA fragments, if we disregard particular bases sequences such as the A-tract [18], is expected to present a small curvature in solution (typically less than 10°), even if larger curvatures have been observed for crystal structures (18° for the Dickerson dodecamer [19]). In our case, the curvature of the NOE structures (about 20°) is much larger than the expected one, and it is significantly reduced by the use of RDC restraints. Indeed, whatever the dilute liquid crystal used, the accepted structures present small curvature and are close together. This result, associated to the symmetry property of the sequence, indicates that whatever the orienting media, the use of RDC allows the improvement of solution structures, as already observed for different DNA or RNA fragments [13,14,20]. This is an important result, since the interactions between DNA and the different mesogens used in this study vary by the charge repartition from the negatively charged bicelles/SDS and phages to the neutral PEG, or by the overall shape of the mesogens (bicelles, rod or concentric cylinders, respectively). This reinforces the idea that the risk of modification of conformations or conformational equilibria by the nearby presence of mesogens is small for this type of molecule. The present work validates our experimental procedure for using RDC to determine the overall shape of DNA. This strategy will be applied to the determination of DNA duplex containing lesion in the sequence.

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