Theoretical NMR study of the pre-melting transition in the d-(CGCGAATTCGCG) and d-(CGCGTATACGCG) self-complementary duplexes

C. Giessner-Prettre and B. Pullman*

Institut de Biologie Physico-Chimique, Laboratoire de Biochimie Théorique associé au CNRS, 13, rue Pierre et Marie Curie, 75005 Paris, France

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The difference between their shielding in a B-DNA duplex and in the single strand having the same conformation has been calculated for all base protons of the dodecamers d-(CGCGAATTCGCG) and d-(CGCGTATACGCG). The calculated chemical shift variations reproduce the qualitative features of the shifts which occur during the pre-melting of the helices. This agreement shows that the pre-melting mechanism consists mainly of a lengthening of the hydrogen bonds between the two strands (in line opening) and that the conformation of the ribophosphate backbone and the orientation of the bases do not undergo major modifications during the first step of the melting.

NMR pre-melting shifts *Hydrogen bond length*

1. INTRODUCTION

Conformation in solution of the double helices formed by self-complementary oligonucleotides has been studied by several spectroscopic methods, including proton NMR ([1–4] and references therein). However, little attention has been paid to the mechanism which initiates the helix–coil transition. Some ^{1}H NMR data concerning the pre-melting of such short helices have been recorded [5–7]. They can be used to determine the conformational parameter of the helix which is first modified when the temperature of the solution increases.

In an attempt to obtain information on the mechanism of pre-melting of two dodecamers investigated experimentally, namely d-(CGCGAATTCGCG) [5] and d-(CGCGTATACGCG) [6], we have undertaken the calculation of the magnetic shielding of all the distinct base protons of the double helix having the conformation found to be the most probable in solution and analogous computations for the 'modified' helix. The comparison between the calculated and observed chemical shift variations should indicate the probable nature of the helix distortion at the start of the process. The choice of these two particular dodecamers is mainly due to the striking difference of the pre-melting shifts measured for the adenine H2 protons of the central AT basepairs (fig.1,no.6). The variations are several times larger for those of the TATA sequence than for those of the AATT one, while the chemical shifts of the other base protons of the 2 molecules undergo similar variations during the pre-melting transition. Therefore the calculation of a different or a similar chemical shift variation according to the AATT or TATA sequence will give a good criterium for the quality of the theoretical results.

2. RESULTS AND DISCUSSION

The computations were done by the modified version of the SHIFTS program [8], used in our study of the solution conformation of Dickerson's

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* To whom correspondence should be addressed

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dodecamer d-(CGCGAATTCGCG) [9].

For the two dodecamers we report in table 1, under the heading dσ, the shielding (ring current effect plus local magnetic anisotropy contributions) produced on each base proton, by every unit of the duplex except the base to which it belongs. We report the values concerning only the 6 basepairs which appear distinct in the experimental ^{1}H spectra (fig.1). The calculation is carried out for the regular B-DNA conformation [10] of the double helices because we have found [9] that it is the most probable one in solution for d-(CGCGAATTCGCG). In addition, the comparison of the shieldings calculated for the two dodecamers shows that the hypothesis of a regular B-DNA conformation for both duplexes appears reasonable since the shielding values are calculated to be larger for the imino protons of the AT pairs of the TATA dodecamer than for the corresponding nuclei of the AATT oligomer, in agreement with [6]. Moreover, for the adenine H2 nuclei the calculated shieldings reproduce the observed reversed order in the two dodecamers [5,6]; namely, AT6 > AT5 in the TATA sequence and AT5 > AT6 in the AATT one [11].

Table 1

Calculated values (in ppm) of the shielding produced by the B-DNA double helix and of the chemical shift variations between the duplex and the single strand

type of proton	bp	d-(CGCGAATTCGCG)		d-(CGCGTATACGCG)	
		dσ[a]	$\Delta\delta$[a]	dσ[a]	$\Delta\delta$[a]
N1H(G)	1	- 0.59	0.78	- 0.59	0.21
	2	- 0.08	0.28	- 0.08	0.22
	3	0.15	0.06	0.14	0.35
	4	0.22	0.63	0.59	0.33
N3H(T)	5	- 0.19	0.96	0.84	2.14
	6	0.74	1.08	1.40	2.61
H2 (A)	5	1.31	- 0.11	1.21	- 0.76
	6	0.84	- 0.13	1.27	- 0.82
H5 (C)	1	0.28	0.08	0.10	- 0.02
	2	0.43	0.06	0.45	0.06
	3	0.53	- 0.01	0.50	0.01
	4	0.34	0.04	0.59	0.01
Me (T)	5	0.28	0.07	0.20	0.02
	6	0.39	0.09	0.33	0.
H6 (C)	1	0.13	0.01	0.13	0.
	2	0.17	0.03	0.18	0.05
	3	0.25	0.01	0.20	0.04
	4	0.16	0.05	0.26	0.02
H6 (T)	5	0.22	0.08	0.23	0.02
	6	0.26	0.07	0.25	0.03
H8 (G)	1	0.14	0.01	0.14	0.02
	2	- 0.22	0.02	0.22	0.03
	3	0.23	0.05	0.28	0.05
	4	0.30	- 0.02	0.26	0.04
H8 (A)	5	0.31	- 0.01	0.30	0.02
	6	0.35	0.02	0.26	0.03

[a] Positive values correspond to increased shielding and upfield chemical shift variations

Fig.1. Numbering of the basepairs used for the two dodecamers.

The upfield shifts measured, during the pre-melting, not only for the imino protons of the AT pairs but also to a smaller extent for those of the GC pairs of one of the dodecamers suggest that the lengths of the pairing hydrogen bonds increase with temperature. The semi-empirical [12] as well as ab initio [13] theoretical studies have clearly illustrated the drastic effect of the intermolecular distance on the variation of chemical shift due to hydrogen bonding.

To simulate the chemical shift variation due to an 'in line opening' of the basepairs we have calculated the shielding produced on the base protons by all the units of a single stranded dodecamer, kept in the regular B-DNA conformation as in the duplex.

For a given proton the difference between its shielding in the duplex and in the single strand yields the shielding due to the second strand of the duplex; it is the upper limit of the chemical shift variation due to an increase of the pairing hydrogen bond length since it corresponds to an infinite distance between the two strands. The values of these differences are reported under the heading $\Delta\delta$ in table 1. They are expected to reproduce the qualitative trends of the modifications of the spectra which occur during the pre-melting, that is the direction of the observed shifts and the relative magnitude of the variations measured for the different protons studied. We must, however, be aware that such calculations cannot reproduce the numerical value of the chemical shift variations since the hydrogen bond length has a finite value when the cooperative melting occurs while the calculated values of $\Delta\delta$ correspond, as already stated, to an infinite interstrand distance.

If we consider the imino protons we observe that for the 6 distinct basepairs of the two dodecamers the calculated values of $\Delta\delta$ are positive (upfield) as are the shifts of the resonance lines of these protons during the pre-melting transition [5,6]. Moreover, the calculated $\Delta\delta$ are larger for the AT pairs than for the GC ones in agreement with the experimental data concerning the d-(CGCGAATTCGCG) dodecamer [5].

For the adenine H2 protons the data in table 1 show that for these protons the calculated chemical shift variations due to the hydrogen bond lengthening is downfield in agreement with the measured pre-melting shifts and that for the sixth basepairs it is > 5-times larger for the TATA sequence than for the AATT sequence. This result, which corresponds to the observed shifts for these protons, is due to the fact that the shielding produced on H2 is mainly an intrastrand effect in AATT and an interstrand one in TATA; it is therefore little modified by the removal of the complementary strand in the first case but is very sensitive to the interstrand distance in the second.

The calculated values of $\Delta\delta$ for the other base protons of the molecules have an absolute value < 0.1 ppm and are upfield in all cases with only 4 exceptions. For these protons Patel and coworkers [5] have measured, in the case of the d-(CGCGAATTCGCG) dodecamer, from 20–50°C, the pre-melting temperature range for this duplex, small chemical shift variations, most of them being upfield. In the case of the d-(CGCGTATACGCG) dodecamer the H8 and H6 protons of the AT pairs undergo experimentally small upfield shifts while those of the methyl groups of these pairs are not shifted (AT 5) or shifted very slightly downfield (AT 6) [6].

The comparison of the calculated value of $\Delta\delta$ and the shifts observed between 20–50°C shows that the agreement between theory and experiment is excellent at the qualitative level since the calculations reproduce not only the major trends of the modifications of the spectra but also the differences between the chemical shift variations measured for the two dodecamers (adenine H2) as well as the relative magnitudes of these shifts. This agreement tends definitely to show that the major mechanism of the pre-melting transition consists of the 'in line opening' of the pairing hydrogen bonds or in other words an increase of the interstrand distance within the double helix [14]. The present results do not exclude the occurrence of some twisting of the base and/or unwinding of the helix [5,6] but the small magnitude for the H8, H5, H6 and 5-methyl protons of the $\Delta\delta$ measured and of those calculated with the hypothesis that the ribophosphate backbone conformation and the orientation of the bases is retained during the pre-melting transition show that the torsion angles of each strand undergo only minor changes during this phase of the helix–coil transition.

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