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A structural study of phosphate-methylated d(CpG)_n and d(GpC)_n DNA oligomers. Implications of phosphate shielding for the isomerisation of B-DNA into Z-DNA

Peter J. L. M. Quaedflieg, Leo H. Koole, Marcel H. P. van Genderen and Henk M. Buck*

Department of Organic Chemistry, Eindhoven University of Technology, P.O. Box 513,
5600 MB Eindhoven, The Netherlands

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Abstract. It is shown that the phosphate-methylated DNA miniduplex d(CpG)₂ adopts a left-handed Z-type structure, whereas the phosphate-methylated miniduplex d(GpC)₂ is right-handed. Furthermore, it is shown that elongation of the systems to the tetramer level results in right-handed duplexes with rather low stability. The phosphate-methylated hexamer d(CpGpCpGpCpG) is present in the single-strand form. It is suggested that these results are of interest in the understanding of the exact role of salt cations in stabilizing the Z-DNA structure of natural duplexes d(CpG...CpG)₂ in high salt solution, since the methylated phosphate groups in fact mimic the situation of complete phosphate-charge shielding. Therefore, it is concluded that exclusive cation-phosphate complexation in the d(CpG)₂ segments stabilizes the Z structure. ³¹P-NMR experiments on the natural duplex d(CpGpCpGpCpG)₂ at different concentrations of Mg²⁺ provided independent proof for the proposed model.

Introduction

The salt-induced transition from right-handed B-DNA into left-handed Z-DNA¹ clearly illustrates the structural versatility of the DNA double helix. While the conformational features of Z-DNA are known in detail from numerous spectroscopic and X-ray diffraction studies²⁻⁵, far less is known about the actual role of salts in stabilizing Z-DNA. Most likely, it is the function of the salt cations to mask the DNA phosphate charges, which are more proximate in Z-DNA than in B-DNA (phosphate-phosphate distance across the helix is 11.9 Å and 17.5 Å for Z- and B-DNA, respectively)². In order to study the impact of phosphate-charge shielding in greater detail, we have chosen the phosphate-methylated DNA oligomers d(CpG)_n (*n* = 1-3) and d(GpC)_n (*n* = 1, 2). As we have shown previously, methylation of the phosphate groups results in neutralisation of the phosphate charges in such a way that no unfavourable steric interactions are introduced⁶. Alternatively stated, the methylated phosphate group mimics the extreme situation of perfect shielding of the phosphate charge, which generally leads to an increased duplex stability with respect to natural duplexes⁷⁻⁹.

Results and discussion

The phosphate-methylated dinucleotides d(CpG) and d(GpC) were studied in diastereomerically pure form⁹ using high-field ¹H-NMR (600 MHz, 14.0 Tesla), circular dichroism (CD) spectroscopy and UV hyperchromicity. The ¹H-NMR spectra in H₂O at 4°C showed a single imino

resonance in the low-field region (14.0-14.5 ppm), proving the formation of two symmetry-related G-C base pairs in each case. Concerning the phosphate-methylated duplex d(CpG)₂, it was found, via variable-temperature ¹H-NMR (see Fig. 1) and UV-hyperchromicity experiments, that a reversible duplex ⇌ coil (melting) transition occurs at *T*_m values of 9°C and 13°C for the *R*_p and *S*_p diastereomers, respectively.

Furthermore, both d(CpG)₂ miniduplexes adopt the left-handed Z-geometry¹⁰. This was obvious from the ¹H-NMR data concerning the conformational properties of the sugar-phosphate backbone (see Table I and Fig. 2). C2'-*endo* and C3'-*endo* puckered sugar rings were found for the dC and dG nucleotide units, respectively, together with a preference for the *g*^t conformation of the C4'-C5' backbone bond in the dG residue². NOE experiments clearly pointed to a *syn* orientation of the G base² and an *anti* orientation of the C base (see Table I). As can be seen from Figure 3, the CD spectra of the d(CpG)₂ miniduplexes also clearly deviate from the right-handed miniduplex structure^{1,11}.

For the counterpart system phosphate-methylated d(GpC)₂, a *T*_m value of 27°C was found (NMR, UV hyperchromicity), both for the *R*_p and the *S*_p diastereomers. Moreover, these miniduplexes are right-handed, as was obvious from the ¹H-NMR conformational data (see Table I) and the CD spectra (see Fig. 3).

The phosphate-methylated tetramer duplex d(CpGpCpG)₂¹³ showed a *T*_m value of 32°C (UV hyperchromicity), while the CD spectrum corresponds with a right-handed double-helix conformation (a positive band was found at 280 nm and a trough at 235 nm)¹¹. The

Fig. 1. Some representative sigmoidal melting curves for R_P and S_P phosphate-methylated $d(CpG)_2$, measured using variable-temperature 1H -NMR. It should be noted that a sigmoidal melting curve was also measured for $H5(C)$ (not shown). For numbering of the protons see Fig. 2.

Table I Some relevant NMR spectral parameters measured for the S_P and R_P phosphate-methylated miniduplexes $d(CpG)_2$ and $d(GpC)_2$, together with the calculated population densities of the $C2'$ -endo pucker of 2'-deoxyribose rings and the $C4'$ - $C5'$ rotamers g^+ and g^- . The most important intramolecular NOE contacts are also summarized. For numbering of the protons see Fig. 2.

Phosphate-methylated $d(CpG)_2$				
	S_P		R_P	
	dCp	pdG	dCp	pdG
$J_{1'2''}$	5.4	8.4	5.0	8.0
$J_{2''3'}$	3.0	9.0	3.4	9.0
$J_{4'5'}$	3.6	4.8	3.8	4.8
$J_{4'5''}$	4.0	9.4	3.8	9.0
$x(C2'-endo)$	0.86	0.04	0.86	0.07
$x(g^+)$	0.61	0.00	0.70	0.00
$x(g^-)$	0.25	0.81	0.26	0.77
NOE contacts	H8-H1'	H8-H2'	H8-H3'	
	0.11	0.00	0.00	
Phosphate-methylated $d(GpC)_2$				
	S_P		R_P	
	dCp	pdG	dCp	pdG
$J_{1'2''}$	5.8	7.2	6.0	7.0
$J_{2''3'}$	2.4	4.0	2.4	4.4
$J_{4'5'}$	3.8	3.0	3.7	2.9
$J_{4'5''}$	3.8	3.0	3.7	2.9
$x(C2'-endo)$	0.88	0.61	0.86	0.59
$x(g^+)$	0.61	0.77	0.63	0.79
$x(g^-)$	0.23	0.17	0.22	0.16
NOE contacts	H8-H1'	H8-H2'	H8-H3'	
	0.041	0.031	0.00	

600-MHz 1H -NMR spectrum in H_2O/D_2O (85/15) at $4^\circ C$ showed two imino resonances at 14.32 and 14.43 ppm. This suggests that four G-C base pairs are formed¹⁴, of which the two internal and the two terminal base pairs are related through symmetry. The phosphate-methylated tetramer duplex $d(CpGpCpG)_2$ is found to be rather unstable¹⁵. The counterpart phosphate-methylated tetramer duplex $d(GpCpGpC)_2$ showed a T_m value of $42^\circ C$ (UV hyperchromicity) and a CD spectrum which also clearly points towards a right-handed B-type duplex. The phosphate-methylated duplex $d(GpCpGpC)_2$ is also substantially less stable than expected¹⁵. Most likely, the explanation for the low stability of both phosphate-methylated tetramer duplexes lies in the fact that the $d(CpG)_2$ segments prefer the Z geometry, while the $d(GpC)_2$ units have a preference

Fig. 2. Schematic representation of the primary and secondary structure of phosphate-methylated $d(CpG)$. The top nucleoside (dC) adopts a conformation which is characterized by anti orientation of the C base, $C2'$ -endo pucker of the 2'-deoxyribose ring and g^+ conformation of the $C4'$ - $C5'$ bond. The bottom residue (dG), by contrast, has a syn orientation of the base, a $C3'$ -endo pucker for the 2'-deoxyribose ring and g^- conformation around $C4'$ - $C5'$.

Fig. 3. CD spectra of R_P $d(CpG)_2$ (---), S_P $d(CpG)_2$ (.....), R_P $d(GpC)_2$ (—), and S_P $d(GpC)_2$ (---), recorded in H_2O at $4^\circ C$. These spectra clearly indicate the presence of a left-handed structure for R_P and S_P phosphate-methylated $d(CpG)_2$ and a right-handed structure for R_P and S_P phosphate-methylated $d(GpC)_2$.

for the B geometry (*vide supra*). Apparently, the conflicting structural preferences of the individual $d(CpG)_2$ and $d(GpC)_2$ segments are best accommodated in an overall right-handed duplex structure. We feel that the diminished stability of these duplexes results from forcing the $d(CpG)_2$ units to adopt a right-handed structure. The phosphate-methylated hexamer $d(CpGpCpGpCpG)_2$ was found to be present exclusively in the *single-strand form*. No melting transition could be detected using UV hyperchromicity, and no imino proton signals were found in the

downfield region (12–15 ppm) of the 600-MHz ^1H -NMR spectrum. This result is now understandable, since the duplex of phosphate-methylated $\text{d}(\text{CpGpCpGpCpG})$ would require accommodation of three $\text{d}(\text{CpG})_2$ and two $\text{d}(\text{GpC})_2$ segments, i.e. three $\text{d}(\text{CpG})_2$ units would have to adopt the unfavourable right-handed form.

Based on the present data, we suggest that stabilization of the Z form of natural $\text{d}(\text{CpG}\dots\text{CpG})_2$ DNA duplexes in high salt solution is the result of site-specific shielding of the phosphate groups in the $\text{d}(\text{CpG})_2$ units exclusively. Recent molecular mechanics calculations, focused on the distribution of cations over the surface of Z-DNA, have also shown that complexation will occur with the $\text{d}(\text{CpG})_2$ phosphates but not with the $\text{d}(\text{GpC})_2$ phosphates¹⁶. Finally, we have performed ^{31}P -NMR experiments on the natural hexamer duplex $\text{d}(\text{CpGpCpGpCpG})_2$ at different concentrations of Mg^{2+} . In the absence of Mg^{2+} (corresponding to a B-DNA duplex, as was evident from the CD spectrum), one single ^{31}P -NMR resonance was observed (δ 1.19 ppm). This peak splits into two well-resolved resonances (δ 1.11 and 0.81 ppm; intensity ratio 2:3) at a Mg^{2+} concentration of 2.4 M (the CD spectrum clearly confirmed the presence of the Z-DNA structure). The observation that the Mg^{2+} -induced ^{31}P -chemical-shift change is much larger for the three $\text{d}(\text{CpG})_2$ phosphates (-0.31 ppm) than for the two $\text{d}(\text{GpC})_2$ phosphates (-0.08 ppm) clearly corroborates the fact that Mg^{2+} complexation in Z-DNA predominantly occurs in the $\text{d}(\text{CpG})_2$ units.

Concluding remarks

To the best of our knowledge, these data represent the first experimental clue in understanding the dynamic function of cations in stabilizing Z-DNA. Furthermore, this concept of alternate complexation of the phosphate groups can be used to design novel cationic organic systems (e.g. modified peptides) in such a way that their molecular structure is perfect for stabilizing either the B or the Z structure of natural $\text{d}(\text{CpG}\dots\text{CpG})_2$ duplexes. Research along these lines is currently in progress in our laboratory.

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