Conformation of the phosphate-methylated dinucleotide d(TpA) : synthesis and nuclear Overhauser experiments

Conformation of the phosphate-methylated dinucleotide d(TpA). Synthesis and nuclear Overhauser experiments

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SUMMARY

The phosphate-methylated dinucleotide d(TpA) has been prepared, and the conformation was studied with variable temperature proton NMR spectroscopy. From the observation of an imino proton resonance at 12.5 ppm for sample temperatures lower than 15°C, it can be concluded that a mini duplex is formed. Analysis of the NOE contacts shows that H_8 of adenine resides in the vicinity of $H_{1'}$ and $H_{2'}$. The couplings $J_{1'2'}$ and $J_{1'2''}$ are approximately 6.5 Hz, which points towards South conformations of the sugar ring in the dT and dA residues. Collectively, these data show that the duplex of phosphate-methylated d(TpA) does not have the Z geometry (which was found for phosphate-methylated d(CpG), see Moody et al., 1987). The present mini duplex shows a marked flexibility which allows syn ⇄ anti-equilibration of the adenine base.

The conversion from right handed B DNA into left handed Z DNA in doublestranded natural DNA with a $d(GC)_n$ nucleotide sequence (G: guanine; C: cytosine), can be induced by increasing the salt concentration (i.e., LiCl, LiBr, KCl, $MgCl_2$), or by changing the solvent from water into aqueous ethanol (Preisler, 1987, Saenger, 1984). The most effective stabilizers of the Z conformation are cations with a small radius and a highly positive charge (e.g., Li^+, Mg^{2+}). Furthermore, it is known that chemical modification of the DNA may also facilitate the B into Z transition for $d(GC)_n$. In particular, this holds for substitution at C_5 of cytosine. For example, methylation at C_5 of cytosine in $d(GC)_n$ leads to a B→Z conversion at considerably lower salt concentrations, in comparison with natural $d(GC)_n$.

Very recently, we have shown that methylation of the phosphate group in the

dinucleotide d(CpG) also results in the Z DNA structure, even in a salt free aqueous solution (Moody et al., 1987). The Z geometry was inferred from a particularly strong nuclear Overhauser (NOE) contact between H_8 and $H_{1'}$ of the guanine residue. This strongly points towards a syn conformation of the guanine base with respect to its own deoxyribofuranose ring. The syn conformation of guanine in $d(GC)_n$ is essential for the accommodation of the Z geometry. Inspection of the Z DNA stucture (Wang et al., 1981) clarifies that the Z geometry is only feasible for alternating purine-pyrimidine sequences. On the other hand, it is not at all clear why $d(GC)_n$ is exceptional in its ability to adopt the Z form. Several investigations have been directed towards detection of the Z form in $d(AT)_n$ (A: adenine; T: thymine).

It can be concluded that $d(AT)_n$ sequences do not adopt the Z conformation unless some exceptional constraint is exercised. An example of this is the decamer d(CGCGATCGCG) in wich the cytosines are brominated at C_5. A single crystal X-ray diffraction study of this system has disclosed a left handed geometry, in which the T bases are fixed in a syn orientation (Feigon et al., 1985).

Herein, we wish to report the preliminary results of a structural study on the phosphate-methylated dinucleotide d(TpA) (figure 1). It is found that this system is present as a duplex structure in aqueous solution, provided that the sample temperature does not exceed approximately 15°C.

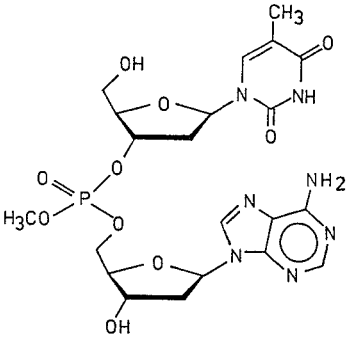

Fig. 1. Structural formula of phosphate-methylated d(TpA).

This conclusion is based on the fact that an imino proton signal is observed at a chemical shift of 12.5 ppm in the high resolution proton NMR spectrum, provided that the sample temperature is lower than 15°C (See: Experimental Section). Analysis of the NOE contacts in phosphate-methylated d(TpA) at 15°C revealed that H_8 of adenine resides in the vicinity of both $H_{2'}$ and $H_{1'}$. The NOE contact between H_8 and $H_{2'}$, points towards the anti-orientation of adenine, whereas the $H_8 \cdots H_{1'}$ NOE contact demonstrates that adenine also resides in the syn domain. At a sample temperature of 2°C, it is found that the $H_8 \cdots H_{1'}$ NOE is slightly stronger than the $H_{8'} \cdots H_{2'}$ NOE. Comparison

with the NOE data on phosphate-methylated d(CpG) in which the $H_8 \cdots H_{1'}$ NOE was exclusively present, reveals that phosphate-methylated d(TpA) does not adopt the Z geometry in solution. Instead, it is likely that a flexible duplex is present, in which the adenine bases are more or less free to rotate around the glycosidic bonds. From the NMR spectrum at 2°C it is also apparent that the coupling constants $J_{1'2'}$ and $J_{1'2''}$ are both approximately 6.5 Hz. These data show that the furanose rings in the dA and dT residues are predominantly in the South conformation. For Z DNA, it is expected that the dA furanose ring is North ($C_{3'}$-endo/$C_{2'}$-exo), while the dT furanose ring is South ($C_{2'}$-endo/$C_{3'}$-exo).

EXPERIMENTAL SECTION

Phoshate-methylated d(TpA) was synthesized on an Applied Biosystems 381 A DNA synthesizer. In order to obtain the phosphate-methylated structure, the standard phosphoramidite synthesis protocol was substantially modified. The synthesis was started with a commercial column, which contains 15 μ mol 5'-O-tritylated thymidine residues immobilized on a controlled pore glass matrix. After detrytilation, a routine coupling with the 5'-O-tritylated, N_6-protected 2'-deoxyadenosine 3'-O-methoxyphosphoramidite was performed.

Subsequently, the phosphite triester was not oxidized. Instead, the column was removed from the synthesizer, and decoupling and deblocking of the substrate with concentrated ammonia was performed. This yielded a basic solution of the 5'-O-tritylated dinucleoside phosphite triester (figure 2). After neutralization of the solution, the phosphite was oxidized with I_2/lutidine (Herdering et al., 1985).

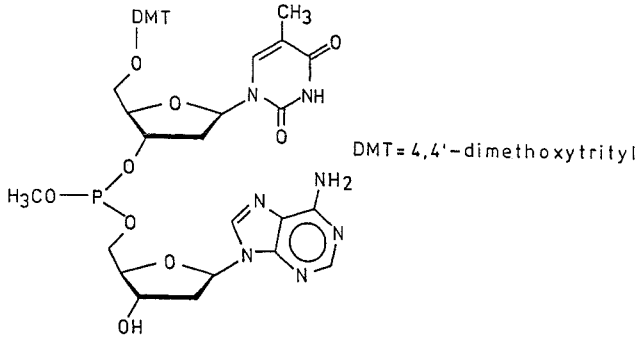

Fig. 2. Structural formula of the 5'-O-protected dinucleoside phosphite triester intermediate.

Deblocking of the 5'-OH site was accomplished with 80% acetic acid (Pon et al., 1985). Finally, the product was purified with two-dimensional preparative thin layer chromatography, using 20% methanol in chloroform as eluent in both directions. NMR spectra were recorded on a Bruker AC 200 NMR spectrometer. Detection of the imino proton resonances was performed

on a solution of phosphate-methylated d(TpA) in 1:1 H_2O/D_2O solution. A standard solvent suppression technique was used to suppress the residual HDO peak in the centre of the spectrum.

All NOE experiments were performed in D_2O solution.

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