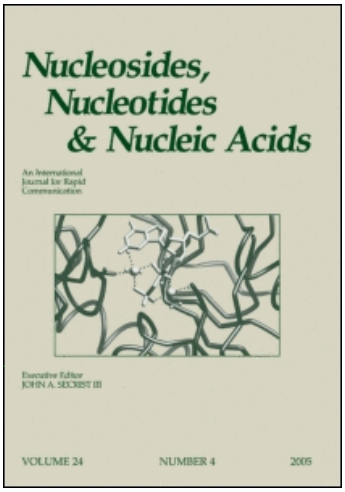

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Interaction Between the Guanine Amino Group and the Adenine Six Membered Ring Stabilizes the Unusual Conformation of the CpA Step in B-DNA

J. Šponer[a]; J. Jursa[a]; J. Kypr[a]
[a] Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic

To cite this Article Šponer, J. , Jursa, J. and Kypr, J.(1994) 'Interaction Between the Guanine Amino Group and the Adenine Six Membered Ring Stabilizes the Unusual Conformation of the CpA Step in B-DNA', Nucleosides, Nucleotides and Nucleic Acids, 13: 8, 1669 — 1677
To link to this Article: DOI: 10.1080/15257779408009472
URL: http://dx.doi.org/10.1080/15257779408009472

NUCLEOSIDES & NUCLEOTIDES, 13(8), 1669-1677 (1994)

INTERACTION BETWEEN THE GUANINE AMINO GROUP AND THE ADENINE SIX MEMBERED RING STABILIZES THE UNUSUAL CONFORMATION OF THE CpA STEP IN B-DNA

J. Šponer, J. Jursa and J. Kypr
Institute of Biophysics, Academy of Sciences of the Czech Republic,
Kralovopolská 135, CZ-61265 Brno,
Czech Republic

Abstract: The CpA step is dramatically overwound in several B-DNA oligonucleotide crystal structures and its AT pair is substantially shifted towards the cytosine of the preceding base pair and towards the minor groove. We show using a geometrical analysis of the crystal data and empirical potential calculations that a strong interaction between the guanine amino group and the adenine six membered ring is responsible for the unique conformational properties of the CpA step.

Introduction

Single crystal X-ray diffraction analysis of DNA fragments provides unique structural information which permits to study how the DNA double helix structure depends on the base sequence. The CpA steps exhibit a very high twist (even higher than 50^{o}), an extremely high slide (approximately 2.6 Å) and a negative roll in the isostructural monoclinic B-DNA d(CCAxxxxTGG)$_2$ decamers[1-4]. Similar, although less pronounced features have been observed at the two independent C2pA3 and T8pG9 steps of the d(CCAGGC^{5m}CTGG)$_2$ decamer exhibiting quite different crystal contacts[5] while the unusual conformation of the CpA and TpG steps is encountered neither in the trigonal d(CCAACITTGG)$_2$ B-DNA decamer[4] nor the B-DNA dodecamers.

The unusual CpA steps exhibit base stacking with the exocyclic carbonyl oxygens and amino groups stacked atop the aromatic rings of the neighboring bases[5]. Similar stacking was observed in many crystals of nucleic acid constituents[6] and explained to result from the induction forces, i.e. interactions of permanent dipoles with delocalized electrons of the aromatic bases. The same mechanism has

been proposed to stabilize the unusual CpA step[2]. However, there are reasons to question this widely accepted explanation.

Firstly, no significant induction or polarization contributions follow from the quantum chemical studies of the base stacking[7-9]. Secondly, the stacking observed in the crystals of small compounds is dramatically affected by the crystal packing forces and hydrogen bonding[10,11]. Finally, the interaction of exocyclic groups with the DNA base rings and a poor base overlap is a rather common feature of the alternating pyrimidine-purine steps, not only of the unusual CpA step[12]. Therefore neither the existing experimental data nor the theoretical studies substantiate the explanation based on the induction forces for the anomalous behavior of the CpA steps in the oligonucleotide crystal structures. Here we propose a different mechanism for the unusual deformability of the CpA step in B-DNA.

Method

We took the geometry of the C2pA3 step of the $d(CCAGGCCTGG)_2$ decamer[2] (the PDB data file 1BD1) and generated all other 15 DNA steps to have the same geometry as the CpA step. Then we looked (the look is very informative, see below) at the base pair overlap in all 16 steps and calculated the base stacking energies using the 6-9 Lifson-Hagler (6-9LH) set of the van der Waals parameters[13]. This force field was chosen because it reproduces the base pair step geometries observed in the highly resolved DNA crystal structures[14-16]. The 6-9LH does not have any consistent set of the atomic partial charges for the DNA bases. Therefore the 6-9LH van der Waals parameters were combined with the AMBER set of partial atomic charges[17] in the present study. These charges are quite reliable because they were obtained independently of the other terms of the force field through quantum mechanical calculations[17]. The solvent effects were included using the Hingerty screening function[18] which, in contrast to many other distance dependent dielectric models, agrees with studies of the base stacking energies, based on the solution of the Poisson-Boltzmann equation[19]. The dependences of the base stacking energy of all 16 steps on the base pair slide and shift were scanned in detail. The base pair vertical separation was optimized in each geometry because its fixation was

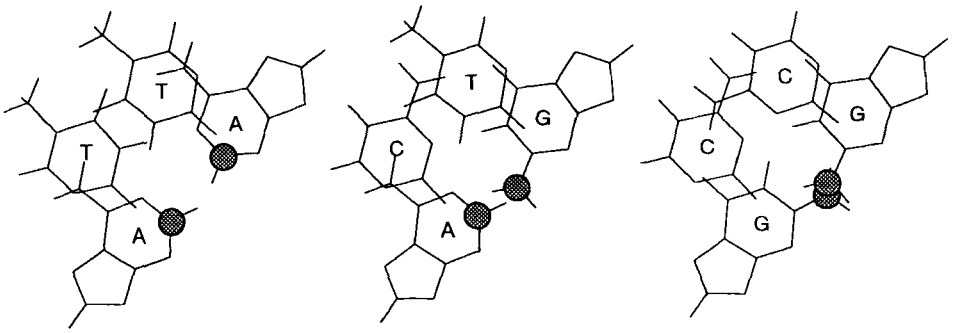

FIG.1: The TpA (left), CpA (middle) and CpG (right) steps in the high twist - high slide - negative roll geometry observed in the crystal structure of the CpA step of the $d(CCAGGCCTGG)_2$ decamer (1BD1). The black circles indicate the purine N2 amino groups and the C2 carbons whose contacts decide about the conformations the steps prefer. For details see the text.

shown to give incorrect results[14-16]. Twist, tilt, roll and the base pair parameters including propeller were kept fixed at the crystal structure values. However, their effects were estimated by repeating the calculations with various different fixed values of these parameters.

Results

Geometrical analysis

FIG.1 shows the pyrimidine-purine steps, having the same geometry as the unusual CpA step. In the case of the TpA step, the two adenines are separated by a large gap giving rise to no interstrand base overlap. This gap is filled by the guanine amino group with the CpA step. Thus, in contrast to the TpA step, the stabilizing interstrand purine-purine dispersion interaction becomes non-negligible. The purines are mutually inclined by about 15-16^{o} at the minor groove side due to both propeller (-8^{o}) and roll (-7^{o}, see below). The guanine amino group points towards the six membered ring of adenine, mainly toward the CH group in position 2 (FIG.2) and the N2(G)-C2(A) distance is only 3.3 A. Slide cannot be decreased in this geometry because that would lead to even a shorter distance between the N2(G) amino group and

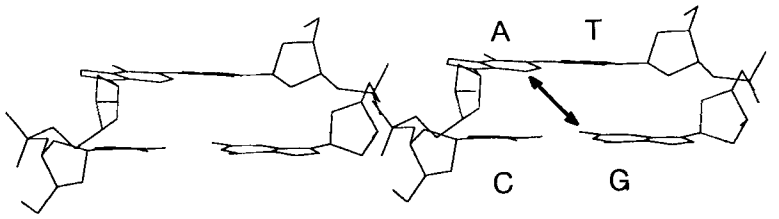

FIG.2: Stereoscopic view of base stacking in the CpA step of the $d(CCAGGCCTGG)_2$ decamer. Close contact of the guanine amino group with the six membered ring of adenine is indicated by an arrow.

the adenine six membered ring. The very strong short range repulsive forces prevent this manoeuvre unless the other conformational parameters are significantly changed. On the other hand, the slide can partially be decreased in the TpA step but only until the six membered rings of the two adenines clash. Nevertheless, a much smaller slide can be accepted in the TpA step as compared to the CpA step due to the amino group absence on the minor groove side.

The CpG step, having two amino groups in the minor groove, cannot adopt the CpA conformation because the amino groups of guanines would clash (FIG.1). This step requires slide of about 3.5 Å to eliminate the clash, which would lead to an unacceptable extension of the sugar phosphate backbone (see below) and a very poor overlap of the bases.

The base pair overlap is poor with the remaining steps in the high twist - high slide geometry (not shown)- and we could find no mechanism to stabilize their high twist - high slide geometry.

The high twist - high slide conformation of the B-DNA steps significantly extends the sugar phosphate backbone[1,20]. It forces the step to adopt a negative roll to diminish the backbone extension[1,20]. The observed roll of the unusual CpA steps ranges from -7^{o} up to -16^{o}[1,4]. Therefore, the mechanism stabilizing the observed unusual CpA high twist - high slide - negative roll geometry can be summarized as follows:

i) The high helical twist makes the CpA step adopt the high slide conformation to remove the minor groove steric contact between the guanine and adenine.

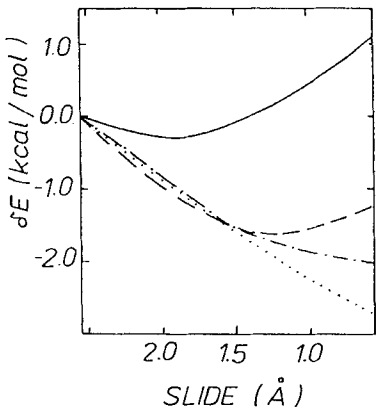

FIG.3.
Dependences of the base stacking energy on slide for the _____ CpA, - - - - - - - - TpA, -.-.-.-.- ApT and ..... ApA steps in the overwound geometry of the CpA step. Values of δE (the base stacking energy in the given conformation minus the base stacking energy in the initial high slide conformation) characterize the relative stability of the high slide geometry.

ii) The extended backbone conformation leads to negative roll, tilting the guanine amino group towards the adenine which further stabilizes the high slide.

iii) Simultaneously, the base stacking is stabilized by a dispersion interaction between the guanine amino group and adenine.

Empirical potential calculations

Stability of the high slide - high, twist - negative roll conformation of the 16 steps was further analyzed using empirical potential calculations. FIG.3 shows the calculated changes in the base stacking energy, caused by reduction of slide, for the CpA, TpA, ApA and ApT steps (the remaining purine-purine, purine-pyrimidine and pyrimidine-pyrimidine steps exhibit similar energy dependencies as the ApA or ApT steps). The CpA (and TpG) step has, in the overwound crystal geometry, a broad base stacking energy minimum with respect to slide (FIG.3) and shift (not shown). However a large decrease of slide is not

allowed due to the potential clash between the guanine amino group and the adenine six membered ring (see Figs. 1 and 2). In contrast, the other steps are unstable in this geometry and tend to decrease slide by about 1.0 Å (TpA) and 1.5-3.0 Å (the remaining 13 steps). This decrease of slide leads to a significant base stacking energy stabilization by 1.6-3.0 kcal/mol. The van der Vaals contribution to the base stacking energy dominates the stabilization, while the energy changes due to the electrostatic term are much less important. We recall that the extreme local overwinding of the B-DNA double helix (helical twist higher than 50^{o}) is absolutely essential for the specific tolerance of the CpA (TpG) step to the big slide. Our calculations for helical twist of 44^{o} indicate that the slide of the CpA step decreases by 0.5-1.0 Å, which is in an excellent agreement with the less twisted geometries of the CpA (TpG) steps in the $d(CCAGGC^{5m}CTGG)_2$ decamer.

For roll 0^{o}, the CpA step partially loses its prominence, though the energy gained by decreasing the slide to typical B-DNA values is still smaller than for the remaining steps (not shown).

We also analyzed effects of modifications of the CpA step by systematic removing the base exocyclic groups. These calculations confirmed the significance of the amino group of G, while the other exocyclic groups had no substantial effect on the relevant van der Vaals energy hypersurface. FIG.4 shows the interplay between the helical twist and the optimized base overlap for the hypothetical pyrimidine-purine step having no exocyclic groups. Even in this case and in contrast to the purine-purine and purine-pyrimidine steps (not shown), an increased twist leads to a stacking geometry with non-zero slide (note the almost invariant position of the six membered purine ring stabilized mainly by the purine mutual inclination). This result agrees with the crystal data, showing larger than the average values of slide with the pyrimidine-purine steps in B-DNA[21]. The amino group at position 2 of one purine base (but not both) in the pyrimidine-purine step increases the distance between the purine bases even more, as it is observed in the unusual CpA step geometry (FIG.1).

Discussion

We demonstrate that the unusual geometry seen in several B-DNA oligonucleotide crystal structures, where the local helical twist and

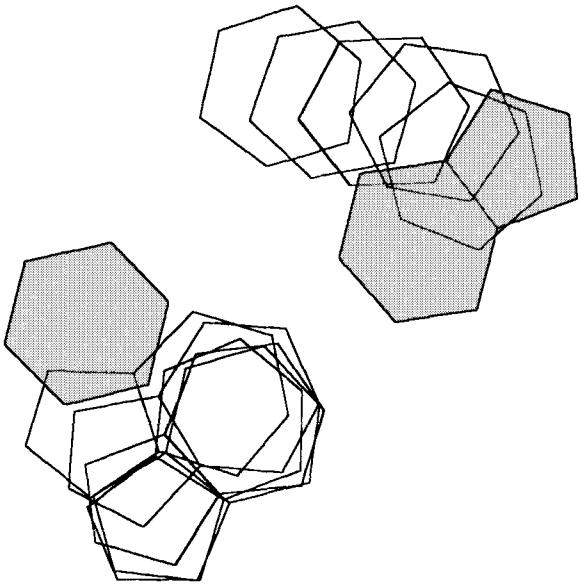

FIG.4: "Animation" of how the base pair overlap (shift and slide) depends on the helical twist in a hypothetical pyrimidine-purine step with no exocyclic groups. Helical twist is 52^{o}, 45^{o}, 36^{o}, 25^{o} and 13^{o}, the base pair vertical separation, shift and slide are optimized and the remaining step and base pair parameters are fixed at the values of the overwound CpA crystal geometry. The bottom (shaded) pair is fixed.

slide are clearly outside the values encountered with canonical DNA structures, is a property which is specifically connected with the CpA (TpG) step. Its native conformation is B-like but the CpA (and partially the TpA) steps differ from the other steps by their deformability. When an external force is applied, the CpA step easily deforms to adopt the unusual conformation observed in some oligonucleotide crystal structures. The interaction of the guanine amino group with the six membered ring of adenine significantly contributes to the stability of the unusual conformation having the high twist - high slide - negative roll geometry. Therefore stresses caused by crystal packing, supercoiling, or proteins bound to the sequences containing TpG (CpA) steps, can be relieved by a conformational transition from B-DNA into the high twist - high slide conformation specifically at the TpG (CpA) steps to give rise to the conspicuous break and shift of the double helix axis observed in the crystal structures. This might be an efficient mechanism of information

transmission from the nucleotide sequence to the higher structures of DNA and its interactions with proteins. Indeed, DNA binding sites of proteins often contain TpG (CpA) steps[22,23]. It is also interesting that the human genome contains many conserved copies of the $(dT\text{-}dG)_n$ sequence[24]. It might be that this sequence is used to create the desired three-dimensional architecture of the genome within the cell nucleus through its unique deformability.

REFERENCES

1. Privé, G.G., Yanagi, K. and Dickerson, R.E. (1991) J.Mol.Biol. 217, 177-199

2. Heinemann, U. and Alings, C. (1989) J.Mol.Biol. 210, 369-381

3. Privé, G.G., Heinemann, U., Chandrasegaran, S., Kan, L.-S., Kopka, M.L. and Dickerson, R.E. (1987) Science 238, 498-504

4. Lipanov, A., Kopka, M.L., Kaczor-Grzeskowiak, M.. Quintana, J. and Dickerson, R.E. (1993) Biochemistry 32, 1993, 1373-1389

5. Heinemann, U., Alings, C. and Bansal, M. (1992) EMBO J. 11, 1931-1939

6. Bugg, C.E., Thomas, J.M., Sundaralingam, M. and Rao, S.T. (1971) Biopolymers 10, 175-219

7. Rein, R. (1978) Perspect.Quantum Chem.Biochem. 2, 307-362, (Pullman, B., ed.) John Wiley & Sons, NY

8. Langlet, J., Claverie, P., Caron, F. and Boeuve, J.C. (1981) Int.J. Quantum Chem. 20, 299-338

9. Aida, M. and Nagata, C. (1982) Chem.Phys.Lett. 86, 44-46

10. Poltev, V.I. and Shulyupina, N.V. (1986) J.Biomol.Struct.Dyn. 3, 739-765

11. Berkovitch-Yellin, Z. and Leiserowitz, L. (1982) J.Am.Chem.Soc. 104, 4052-4064

12. Yuan, . H., Quintana, J. and Dickerson, R.E. (1992) Biochemistry 31, 8009-8021

13. Lifson, S., Hagler, A.T. and Dauber, P. (1979) J.Am.Chem.Soc. 101, 5111-5121

14. Šponer, J. and Kypr, J. (1991) Theor.Biochem.Mol.Biophys. 1, 271-284 (Beveridge, D.L. and Lavery, R., eds.) Adenine Press, NY

15. Šponer, J. and Kypr, J. (1991) J.Mol.Biol. 221, 761-764

16. Šponer, J. and Kypr, J. (1993) J.Biomol.Struct.Dyn., 11, 277-292

17. Weiner, S.J., Kollman, P.A., Case, D.A., Singh, U.C., Ghio, C., Alagona, G., Profeta, Jr.S. and Weiner, P. (1984) J.Am.Chem.Soc. 106, 765-784

18. Hingerty, B.E., Ritchie, R.H., Ferrell, T.L. and Turner, J.E. (1985) Biopolymers 24, 427-439

19. Friedman, R.A. and Honig, B. (1992) Biopolymers 32, 145-159

20. Yanagi, K., Privé, G.G. and Dickerson, R.E. (1991) J.Mol.Biol. 217, 201-214

21. Heinemanm, U., Alings, C. and Lauble, H. (1990) Nucleosides Nucleotides 9, 349-354

22. Nussinov, R. (1986) Biochim.Biophys.Acta 866, 93-108

23. Schultz, S.C., Shields, G.C. and Steitz, T.A. (1991) Science 253, 1001-1007

24. Pardue, M.L., Lowenhaupt, K., Rich, A. and Nordheim, A. (1987) EMBO J. 6, 1781-1789

Received 5/10/93
Accepted 1/24/94