Conformation of the circular dumbbell d<pCGC-TT-GCG-TT>: Structure determination and molecular dynamics

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Summary

The circular DNA decamer 5'-d<pCGC-TT-GCG-TT>-3' was studied in solution by means of NMR spectroscopy and molecular dynamics in H2O. At a temperature of 269 K, a 50/50 mixture of two dumbbell structures (denoted L2L2 and L2L4) is present. The L2L2 form contains three Watson-Crick C-G base pairs and two two-residue loops in opposite parts of the molecule. On raising the temperature from 269 K to 314 K, the L2L4 conformer becomes increasingly dominant (95% at 314 K). This conformer has a partially disrupted G(anti)-C(syn) closing base pair in the 5'-GTTC-3' loop with only one remaining (solvent-accessible) hydrogen bond between NH of the cytosine dC(1) and O6 of the guanine dG(8). The opposite 5'-CTTG-3' loop remains stable. The two conformers occur in slow equilibrium (rate constant 2-20 s⁻¹). Structure determination of the L2L2 and L2L4 forms was performed with the aid of a full relaxation matrix approach (IRMA) in combination with restrained MD. Torsional information was obtained from coupling constants. Coupling constant analysis (3JHH, 3JHP, 3JCP) gave detailed information about the local geometry around backbone torsion angles β, γ, δ, and ε, revealing a relatively high flexibility of the 5'-GTTC-3' loop. The values of the coupling constants are virtually temperature-independent. 'Weakly constrained' molecular dynamics in solvent was used to sample the conformational space of the dumbbell. The relaxation matrices from the MD simulation were averaged over <r³> to predict dynamic NOE volumes. In order to account for the 1:1 conformational mixture of L2L2 and L2L4 present at 271 K, we also included S² factors and <r⁶> averaging of the <r³>-averaged relaxation matrices. On matrix averaging, the agreement of NOE volumes with experiment improved significantly for protons located in the thermodynamically less stable 5'-GTTC-3' loop. The difference in stability of the 5'-CTTG-3' and 5'-GTTC-3' loops is mainly caused by differences in the number of potential hydrogen bonds in the minor groove and differences in stacking overlap of the base pairs closing the minihairpin loops. The syn conformation for dC(1), favored at high temperature, is stabilized by solvation in the major groove. However, the conformational properties of the dC(1) base, as deduced from R-factor analysis and MD simulations, include a large flexibility about torsion angle γ.

Introduction

Recently it has been shown that DNA sequences known to form two-base loops in vitro also form tight loops in vivo (Davison and Leach, 1994). This ability may play important roles in gene regulation, recombination or mutagenesis.
5' → 3'
T10-p1-C1-p2-G2-p3-C3-p4-T4
| p10 . . . . P5 |
T9 -p5-G8-p8-C7-p7-G6-p6-T5

Scheme 1. Schematic structure of the circular dumbbell I.

Abbreviations: NOE, nuclear Overhauser enhancement; NOESY, nuclear Overhauser enhancement and exchange spectroscopy; HMQC, heteronuclear multiple quantum coherence; HSQC, heteronuclear single quantum coherence; ZQ, zero quantum; IRMA, iterative relaxation matrix approach; rMM, restrained molecular mechanics; rMD, restrained molecular dynamics; wMD, weakly constrained molecular dynamics; vMD, weakly constrained molecular dynamics in vacuo; CBS, complete band-shape method; DSS, 3-trimethyl-silyl-1-propane sulfonic acid, sodium salt; T_m, melting midpoint; ppm, parts per million; °C, arabinofuranosyl-cytidine; mini-hairpin, hairpin with a two-residue loop.