Supporting information

Unique structural features of interconverting monomeric and dimeric G-quadruplexes adopted by a sequence from intron of N-myc gene

Marko Trajkovski[‡], Mateus Webba da Silva[‡] and Janez Plavec^{,‡,§,||}*

[‡]Slovenian NMR center, National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia,

[‡]School of Biomedical Sciences, University of Ulster, Cromore Road, Coleraine BT52 1SA, UK,

[§]Faculty of Chemistry and Chemical Technology, University of Ljubljana, Askerceva cesta 5, SI-1000 Ljubljana, Slovenia and

^{||}EN→FIST Centre of Excellence, Dunajska 156, SI-1000 Ljubljana, Slovenia

Simulated annealing protocol

Simulated annealing (SA) calculations of monomeric and dimeric G-quadruplexes adopted by mycN were performed over 100 ps protocol. 50 structures for both of the G-quadruplex forms were calculated. The force constants were $50 \text{ kcal mol}^{-1} \text{ \AA}^{-2}$ for NOE distance restraints, $50 \text{ kcal mol}^{-1} \text{ \AA}^{-2}$ for restraints corresponding to hydrogen-bonding within G-quartets and $500 \text{ kcal mol}^{-1} \text{ \AA}^{-2}$ for torsion angles restraints. In the first 30 ps the scalar factors of the force constants for NOE distance restraints and restraints corresponding to hydrogen-bonding within G-quartets were held at the initial value of 0.1 and were scaled from the initial to the final value of 1.0 during the subsequent 10 ps. In the case of monomeric G-quadruplex, the scalar factors of the force constants for torsion angles restraints were held for 30 ps at initial value of 0.1 and afterwards scaled during 10 ps to the final value of 1.0. In the case of dimeric G-quadruplex, the scalar factors of the force constants for torsion angles restraints were scaled from the initial value of 0.0 to the value of 0.1 during the first 15 ps, held at this value for 15 ps and afterwards scaled to the final value of 1.0 in the course of 10 ps simulation. Temperature was held at 300 K in the first 30 ps of SA calculations. Protocol included heating over 10 ps from 300 to 1000 K, followed by 20 ps equilibration at 1000 K, cooling over 20 ps from 1000 to 100 K and cooling over 20 ps from 100 to 0 K.

Figure S1. Imino region of ^1H NMR spectrum of N-myc dimeric G-quadruplex at 6.6 mM oligonucleotide concentration per strand, at 100 mM KCl and 20 mM K-phosphate buffer (pH 6.5). a) in 90%/10% $\text{H}_2\text{O}/^2\text{H}_2\text{O}$, b) 1 hour after exchanging solvent to $^2\text{H}_2\text{O}$, c) one week after exchanging solvent to $^2\text{H}_2\text{O}$ and d) after dilution from 6.6 to 2.2 mM oligonucleotide concentration. Signals labeled with * designate imino protons corresponding to monomeric N-myc G-quadruplex.

Figure S2. Native PAGE of N-myc. a) 1.9 mM oligonucleotide concentration per strand. b) Oligonucleotide concentrations were 1.00 mM (lane 1), 0.50 mM (lane 2), 0.25 mM (lane 3) and 0.13 mM (lane 4) per strand. c) Oligonucleotide concentrations were 0.05 mM (lane 1), 0.10 mM (lane 2), 0.06 mM (lane 3), 1.50 mM (lane 4), 3.00 mM (lane 5) and 6.00 mM (lane 6) per strand. The samples were loaded on a gel, which was supplemented with 100 mM KCl (panels a and b) or with 20 mM KCl (panel c). L indicates DNA ladder.

Figure S3. Sedimentation coefficient distributions and the corresponding imino regions of ^1H NMR spectra of identical samples of N-myc at 1.5 mM oligonucleotide concentration per strand. Panels a) and b) at 20 mM K-phosphate buffer (pH 6.5), and panels c) and d) at 100 mM KCl and 20 mM K-phosphate buffer (pH 6.5). Both samples were dissolved in 90% H_2O , 10% $^2\text{H}_2\text{O}$ and data acquired at 25 °C. S-values of 1.6 S and 3.0 S were measured at low K^+ ion concentration (a). S-values of 1.9 S, 2.8 S and 4.2 S were observed at high K^+ ion concentration (b) and correspond to monomeric, dimeric and higher order structures, respectively. Signals labeled with # and * in panels b) and d) designate imino protons corresponding to monomeric and dimeric N-myc G-quadruplexes, respectively.

Figure S4. UV melting profiles of N-myc at different oligonucleotide concentrations, 100 mM KCl and 20 mM K-phosphate buffer (pH 6.5).

Figure S5. CD spectra of N-myc at different oligonucleotide concentrations, 100 mM KCl, 20 mM K-phosphate buffer (pH 6.5) and 25 °C.

Figure S6. a) Imino region of ^1H NMR spectrum of N-myc monomeric G-quadruplex in 90%/10% $\text{H}_2\text{O}/^2\text{H}_2\text{O}$ at 2.7 mM oligonucleotide concentration per strand, 20 mM K-phosphate buffer (pH 6.5) and 25 $^\circ\text{C}$. b) 1D ^{15}N -filtered spectra of 6% residue-specifically ^{15}N -labeled G3, G4, G5, G7, G8, G9, G11, G12, G13, G15, G16 and G17 oligonucleotides. c) Imino region of ^1H NMR spectrum after exchanging solvent to 100% $^2\text{H}_2\text{O}$.

Figure S7. JRHMBC spectrum of N-myc monomeric G-quadruplex with regions showing a) intraresidual correlations of C5 with H1 and H8 for individual guanine residues and b) intraresidual correlations of C5 and C4 with H8, and of C4 and C6 with H2 for adenine residues. Concentrations of oligonucleotide and K^+ ions were 2.0 mM and 20 mM, respectively.

Figure S8. JRHMBC spectrum of N-myc dimeric G-quadruplex with regions showing a) intraresidual correlations of C5 with H1 and H8 for individual guanine residues and b) intraresidual correlations of C5 and C4 with H8, and of C4 and C6 with H2 for adenine residues. Concentrations of oligonucleotide and K^+ ions were 6.6 mM and 120 mM, respectively.

Figure S9. a) Imino region of ^1H NMR spectrum of N-myc monomeric G-quadruplex in 90%/10% $\text{H}_2\text{O}/^2\text{H}_2\text{O}$ at 6.6 mM oligonucleotide concentration per strand, 20 mM K-phosphate buffer (pH 6.5) and 25 $^\circ\text{C}$. b) 1D ^{15}N -filtered spectra of 6% residue-specific ^{15}N -labeled G3, G4, G5, G7, G8, G9, G11, G12, G13, G15, G16 and G17 oligonucleotides. Signals labeled with * designate imino protons corresponding to monomeric N-myc G-quadruplex.

Figure S10. a) Aromatic region of ^1H NMR spectrum of N-myc dimeric G-quadruplex in 90%/10% $\text{H}_2\text{O}/^2\text{H}_2\text{O}$ at 6.6 mM oligonucleotide concentration per strand, 20 mM K-phosphate buffer (pH 6.5) and 25 °C. 2D HSQC spectra of 6% ^{15}N -site specific labeled G7, G13 and G17 oligonucleotides are shown in b), c) and d), respectively. e) Anomeric-aromatic region of NOESY spectrum of N-myc with mixing time of 200 ms at 6.6 mM oligonucleotide concentration per strand, 20 mM K-phosphate buffer (pH 6.5) and 25 °C. Intraresidual H1'-H6/H8 correlations are labeled.

Complete reference 50:

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