CD Spectroscopy of Nucleic Acids

Head
Michaela Vorlíčková

Scientist
Iva Kejnovská

Graduate students
Klára Bednářová, Markéta Fialová, Daniel Renčiuk, Petra Školáková, Martin Tomaško

Group Leader
Jaroslav Kypr

Scientist
Karel Nejedlý

Specialist
Jitka Vondrušková-Motlová

Arrangements of human telomeric DNA quadruplex in physiologically relevant K+ solutions

Telomeres play an important role in cellular aging and cancer. Guanine-rich strands of telomeric DNA form qudraplexes, which are pivotal elements for maintaining telomere integrity and controlling cancer cell proliferation. The arrangement of the human telomeric quadruplex in physiologically relevant conditions has not yet been unambiguously determined. Distinct quadruplex structures were observed by various methods. We have shown that the arrangement of the telomeric DNA is polymorphous: The core quadruplex sequence G3(TTAG3)3 forms an antiparallel quadruplex of a basket type in solution containing either K+ or Na+ ions. Analogous sequences extended by flanking nucleotides (studied in other laboratories) form a mixture of the antiparallel and hybrid, so called (3 + 1), quadruplexes in K+-containing solutions. We have, however, shown that long telomeric DNA behave in the same way as the basic G3(TTAG3)3 motif: They fold into an antiparallel quadruplex structure. Both G3(TTAG3)3 and long telomeric DNA are also able to adopt the (3 + 1) quadruplex structure: Molecular crowding conditions, simulated e.g. by ethanol, induce a slow transition of the K+-stabilized antiparallel quadruplex into the hybrid quadruplex structure and then into a parallel quadruplex. Most importantly, we demonstrate that the same transitions can be induced even in aqueous, K+-containing solution by increasing the DNA concentration. This is why distinct quadruplex structures were detected for AG3(TTAG3)3 by X-ray, nuclear magnetic resonance and circular dichroism spectroscopy: Depending on DNA concentration, the human telomeric DNA can adopt the antiparallel quadruplex, the (3 + 1) structure, or the parallel quadruplex in physiologically relevant concentrations of K+ ions.

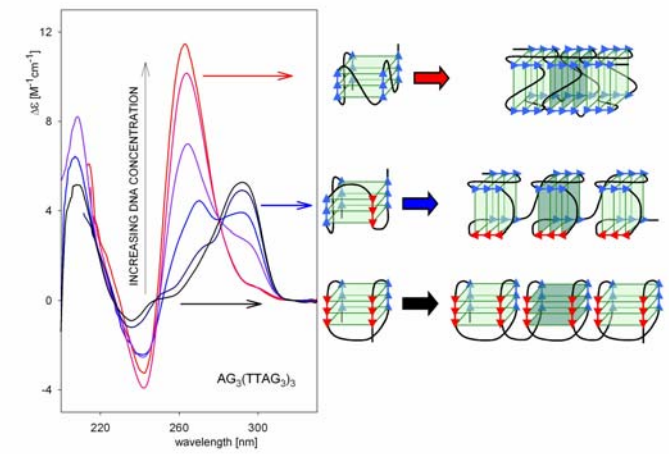

Figure 1: Polymorphism of human telomeric DNA quadruplex. CD spectra corresponding to particular quadruplex arrangements of a human telomeric sequence. On the right there are schematic drawings of particular, concentration dependent quadruplex types of a basic quadruplex unit G3(TTAG3)3 and of a long telomeric DNA chain.

Influence of guanine for adenine mutation in the human G3(TTAG3)3 telomere DNA on its quadruplex folding

DNA of all living organisms is constantly exposed to damages by endogenous oxidation, hydrolysis, and replication errors as well as exogenous genotoxic chemicals and physical agents that lead to mutations. One of frequent spontaneous point mutations is a guanine for adenine conversion. We have studied the formation and structural properties of quadruplexes of the human telomeric DNA sequence G3(TTAG3)3, in which each guanine base was successively replaced by an adenine base. None of these single base substitutions hindered the formation of antiparallel quadruplexes, as shown by circular dichroism, gel electrophoresis, and UV thermal stability measurements in NaCl solutions. Effect of substitution did differ, however, depending on the position of the substituted base. The A-for-G substitution in the middle quartet of the antiparallel basket scaffold led to the most distorted and least stable structures and these sequences preferred to form bimolecular quadruplexes. Unlike G3(TTAG3)3, no distinct structural changes were observed for intramolecular quadruplexes of the A-containing G3(TTAG3)3 analogs when sodium ions were replaced by potassium ions. Their basic quadruplex topology remained the same in both salts. As in vivo missincorporation of A for a G in the telomeric DNA is possible and potassium is a physiological salt, these findings may have biological relevance.

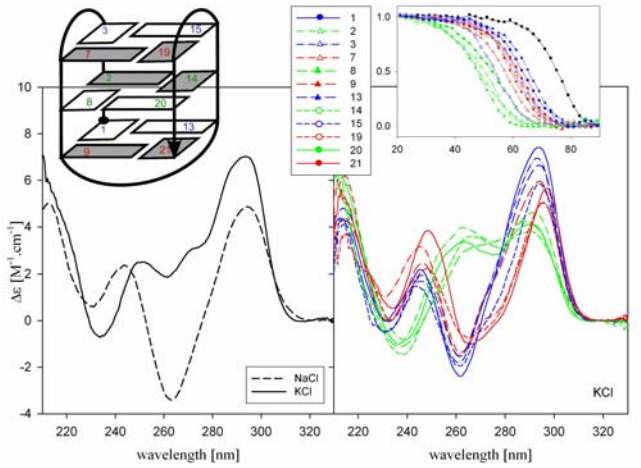

Figure 2: Damage to telomeric DNA sequence by an A for G mutation at particular positions of its quadruplex structure. Left: CD spectra of G3(TTAG3)3 under physiological Na + or K + ions concentrations and a schematic drawing of the G3(TTAG3)3 quadruplex in NaCl (syn- geometries of guanines are shadowed). Right: CD spectra reflecting structures of G3(TTAG3)3 analogs containing A instead of particular G´s and their stability in the presence of K + ions differ from the structure and stability of the intact sequence.

Circular dichroism and conformational polymorphism of DNA

We have reviewed studies that provided important information about conformational properties of DNA using circular dichroic (CD) spectroscopy. The conformational properties include the B-family of structures, A-form, Z-form, guanine quadruplexes, cytosine quadruplexes, triplexes and other less characterized structures. CD spectroscopy is extremely sensitive and relatively inexpensive. This fast and simple method can be used at low as well as high DNA concentrations and with short as well as long DNA molecules. The samples can easily be titrated with various agents to cause conformational isomerizations of DNA. The course of detected CD spectral changes makes possible to distinguish between gradual changes within a single DNA conformation and cooperative isomerizations between discrete structural states. It enables measuring kinetics of the appearance of particular conformers and determination of their thermodynamic parameters. In careful hands, CD spectroscopy is a valuable tool for mapping conformational properties of particular DNA molecules. Due to its numerous advantages, CD spectroscopy significantly participated in all basic conformational findings on DNA.

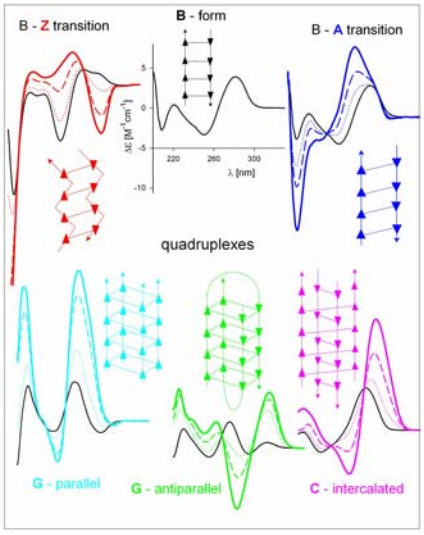

Figure 3: Circular dichroism and conformational polymorphism of DNA. CD spectra of particular DNA arrangements.