Analysis of guanosine quadruplex formation and elongation in dilute aqueous solutions: salt effects

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Guanosine-rich single stranded sequences of DNA, as found in telomeres and in other parts of the genome [1], can adopt various tertiary structures, including G-quadruplexes. G-quadruplexes are four-stranded helical structures, made by the stacking of planar quartets (also indicated as tetramers), arising from Hoogsten hydrogen-bonding between four guanines (G) (see Figure 1). The biological role of such sequences and the structural properties of G-quadruplexes have been extensively discussed [1], and several reviews, focusing mainly on their topology [2] or on telomerase activity [3], have been published. However, the understanding of basic physical properties is still rather limited, even for short sequences comprising only 3 or 4 quartets. In particular, the mechanisms and the principles that govern quadruplex formation and stability in terms of counter-ion effects, as expressed by thermodynamic and kinetic parameters, are still unknown.

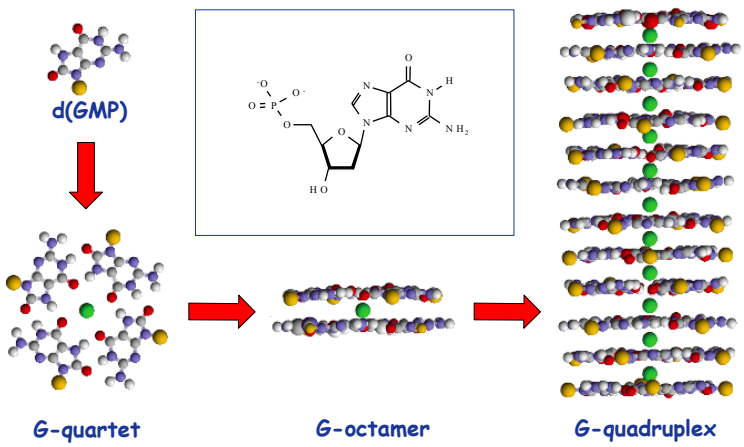

Figure 1: d(pG) aggregates in water (the sugar-phosphate residues and the cations are represented as yellow and green spheres, respectively). The cation is located between two stacked quartets.

Recently, a series of in-solution Small Angle X-ray Scattering experiments on d(pG) have been performed in the presence of different counter-ions at room temperature [4] and as a function of temperature in absence of excess salt, at a concentration just above the critical one at which self-assembling occurs [5]. The obtained results give thermodynamical justification for the observed phase-behavior, indicating that in pure water octamer formation is essential for quadruplex elongation.

The analysis has been extended in 2010, investigating by SAXS at Desy (A2 beamline) the alkalyne cation effects on d(pG) quadruplex elongation and thermal stability. Experiments have been performed as a function of d(pG) concentration using different d(pG) salts and different excess counter-ions. A few results are reported in Figure 2 and clearly indicate that the structural properties of the d(pG) aggregates in solution strongly depend on the experimental conditions. A global fitting approach has been used to derive the composition and size distribution of the scattering particles as a function of composition and temperature. The size and the equilibrium concentration of the different aggregate forms present in solution shows that not only elongation is differently

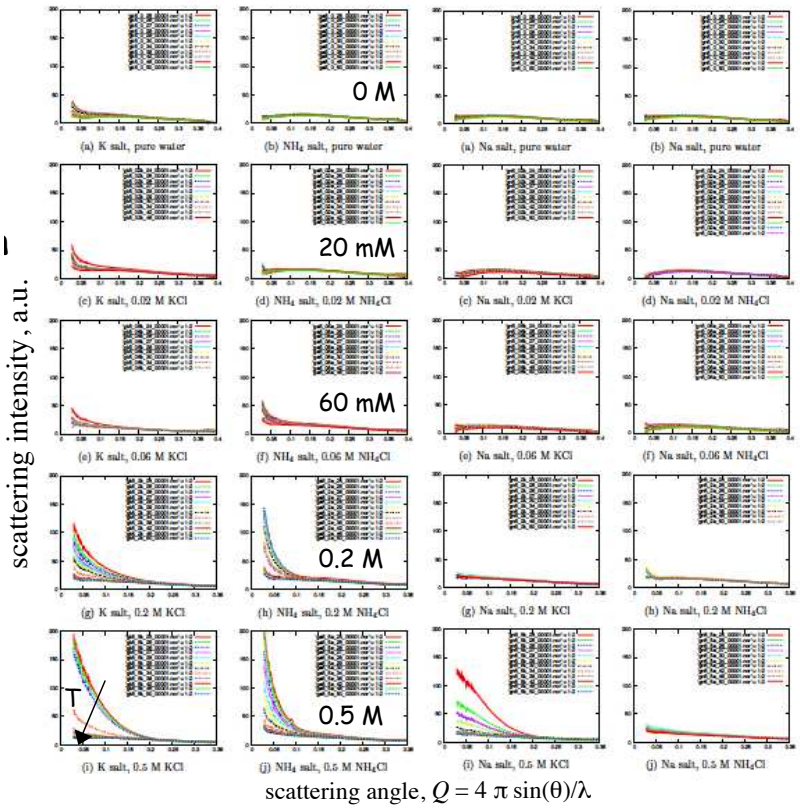

Figure 2: SAXS profile exempla. Column from the left: d(pG) K salt in KCl, d(pG) ammonium salt in NH_4Cl, d(pG) Na salt in KCl, d(pG) sodium salt in NH_4Cl. Salt molarity and temperatures are indicated in the figure.

driven by the different cations (excess potassium is the more efficient, while excess sodium is unable to force quadruplex formation), but also the unfolding temperature strongly depends on the counter-ion concentration and quality. The thermodynamics of the self-assembling process was then analyzed in the framework of a nucleation-elongation model. The unfavorable nucleation step has been clearly identified again with the formation of G-octamers and a hierarchical pathway for the self-assembly of guanosine into helical structures upon cooling proposed. In this framework, the effect of the different counter-ions appears expressed in term of octamer formation equilibrium constant as well as in term of the elongation constants.
Once complete the analysis, we expect to derive a complete description of the d(pG) self-assembling process, that will be used to further clarify the complex mechanism for helix formation, which is critically related to the biological relevance of G-quadruplexes and to the possibility to prepare stable G-based molecular nanowires for nanotechnology applications.

References

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