High pressure studies on G-quadruplex DNA structures

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Short DNA sequences containing several guanine residues can form four-stranded structures called G-quadruplexes (GQ). They have a number of variants, but the common feature is the planar organization of four guanine bases, which are connected by Hoogsten-type hydrogen bond interactions. Two or more of such quartets appear in a GQ. They are mostly stabilized by ions like K^+ and Na^+. Since GQs were found in promoter regions of several oncogenes, they became intensively investigated research objects.

It was found earlier, that the denaturation temperature of GQs can be tuned by pressure [1,2]. The whole pressure-temperature phase diagram like the one of the proteins [3] was however not been determined.

In this study we explored the pressure-temperature behavior of different quadruplex forming aptamers using infrared and fluorescence spectroscopy.

Thrombin binding aptamer (TBA) is a short oligo of 15 bases, which forms a quadruplex structure containing two G-quartets. The human telomere (Htel) sequence is built of three G-quartets.

For the fluorescence experiments we used labeled aptamers. FAM and TAMRA were used for donor and acceptor at the two ends of the aptamer. FRET (Förster Resonance Energy Transfer) was observed in case of folded (GQ structure) aptamers, while the unfolded (single stranded) DNA showed very weak FRET signal. This effect allowed us to obtain the pressure and temperature unfolding profiles.

In the molecular crowding experiments, we used Ficoll and dextrane as crowding agent, to mimic the high concentration of the cell interior.

Although infrared spectroscopy can be used very successfully to determine the molecular conformational changes, there are very few publications about its use for nucleic acids. Combining the FTIR and fluorescence spectroscopy on the same sample, we could identify specific infrared vibrations, which are sensitive to the GQ form.

We determined the temperature stability at different pressure values for both TBA and Htel. Interesting concentration dependence can be observed in the trend of the stabilization-destabilization. Pressure tends to destabilize the GQ-s at low concentrations, while at high concentration we can observe stabilization. The possible sources of these differences (self crowding effect) will be discussed in detail.

A specially constructed setup allowed us to perform simultaneous FTIR and FRET experiments. The infrared and fluorescence spectral changes have a well determined sequence, which allows us to reveal the molecular structural details of the unfolding. Also the thermodynamic and especially the volumetric parameters of the unfolding will be presented. The dissimilarities compared to the protein phase diagram, and the determining factors, like the small compressibility difference, will be also discussed.

Stabilizing GQ by binding different ligands is an important direction in the cancer research. We studied the stabilizing effect of TyMP4, which was found to stabilize the Htel structure.

Another important aspect of the structural changes in the macromolecules is the crowded environment present in the cell. It is known, that macromolecular crowding influences the protein stability [4], but relatively few is known about nucleic acid phase transitions in crowded environment [5].

Our studies revealed pronounced kinetic effects of the crowded environment on the formation of the GQ structure. This effect emphasizes the importance of the excluded volume. The large activation volume of the folding explains that the reduction of the available space in the crowded environment can considerably slow down the folding process.

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