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Self-crowding influences the temperature – pressure stability of the human telomere G-quadruplex

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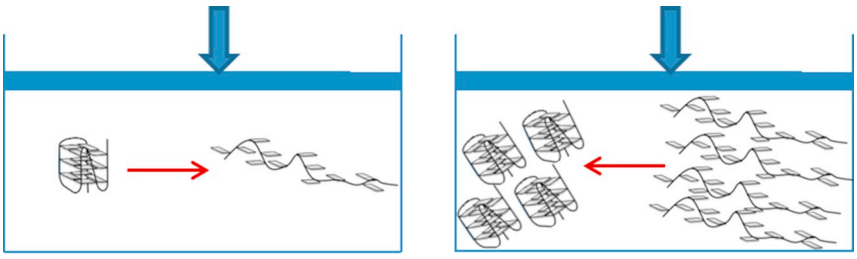
HIGHLIGHTS

- Unfolding volume change of Htel differs in dilute and in self-crowded environments.
- T-p phase diagram of the Htel was determined up to 1 GPa and 20–100 °C.
- The unfolding volume change turns from negative to positive due to self-crowding.
- Appearance of parallel forms might contribute to the sign change of unfolding volume.
- The hydration of the central ions does not decrease the volume in self-crowding case.

GRAPHICAL ABSTRACT



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ABSTRACT

We measured the effect of crowded environment on G-quadruplex structures, formed by guanine rich DNA sequences. Fluorescence and infrared spectroscopy were used to determine the temperature stability of G-quadruplex structure formed by the human telomere sequence. We determined the T-p phase diagram of Htel aptamer up to 1 GPa at different self-crowding conditions. The unfolding volume change was determined from the pressure induced shift of the unfolding temperature of the quadruplex form. The unfolding volume change decreased in magnitude, and even its sign changed from negative (−19 ml/mol) to positive (7 ml/mol) under self-crowded conditions. The possible explanations are the appearance of the parallel GQ structure at high concentration or the fact that the volume decrease caused by the released central K^+ ion during the unfolding is less significant in crowded environment.

1. Introduction

After the discovery of the double helix structure of DNA, several nucleic acid structures were found, which are different from the canonical double helical ones [1]. G-quadruplex (GQ) attracted distinguished attention among the exotic structures, because the sequence typically forming GQ was found at several crucial positions of the genome [2,3]. One example is the telomere region, which is a non-coding sequence at the end of the DNA. This telomere region shortens during each cell division. Shortening of the telomere restricts the possible number of cell divisions. When the telomere region is completely lost, the next cell division will omit base pairs, which code important information, and their loss can lead to the death of the cell. This is called as Hayflick limit. In case of immortal cells, like cancer cells, an enzyme called telomerase elongates the telomere sequence, compensating the shortening and preventing the complete loss of the telomere

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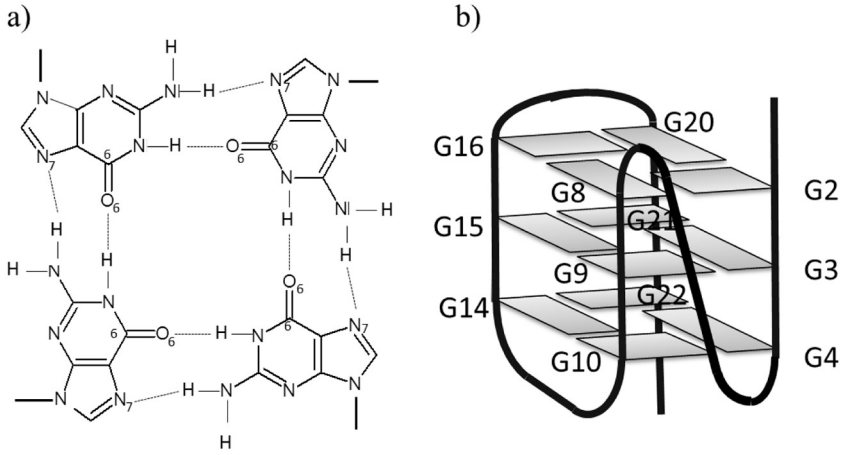

Fig. 1. Structure of the GQ. a) The four guanines are connected by Hoogsteen-type hydrogen bonds. b) Schematic 3D structure of Htel. Drawn following the PDB structures 2HY9 [8] and 2JSM [9].

region [4]. The telomere region in human cells contains typically the TTAGGG repeat [5]. Presence of large number of guanine residues allows formation of GQs. GQs are four-stranded structures, where four guanines are arranged in a plane (called as G-quartet) coupled by Hoogsteen-type hydrogen bonds [6]. GQ is formed by two or three of these quartets (Fig. 1). Generally, the folded form of GQs has to be stabilized by Na^+ or K^+ ions, which are in the middle of the molecule. They are coordinated to the oxygen atoms of the guanine bases [7].

It has been shown, that formation of GQs inhibits the activity of telomerase, which made the quadruplex structures an attractive target for the anticancer treatment. Later quadruplex sequences have been found in several other locations of the genome. Among others they are present in promoters of oncogenes and proto-oncogenes like c-MYC, KIT and VEGF [2,10,11]. This also points to their importance in the tumorgenesis.

One of the mostly studied GQ is the Htel, which originates from the human telomere region. The Htel aptamer is a short GQ forming sequence (AGGG TTAGGG TTAGGG TTAGGG) from the telomere repeat.

GQs can fold in diverse forms. Fujii et al. determined several different folded structures formed by the Htel aptamer in presence of different ions and crowding agents [7]. Htel forms a so called 3 + 1 hybrid type structure (Fig. 1b) in presence of K^+ ion [12]. This means that three of the four strands go downwards and one goes upwards. Fuji et al. found an additional fraction of the 2 + 2 basket forms under these conditions [7]. In case of basket form, both ends of the chain can be found at the same side of the folded GQ, while in the hybrid form the ends are at different sides of the GQ. This gives the possibility to use fluorescence labeling by a FRET pair [13]. Since the distance between the donor and acceptor fluorophores is significantly larger in hybrid form compared to the basket form, it leads to different FRET efficiencies, allowing the distinction between the above forms.

Although several experiments have been performed to characterize the stability and structural behavior of GQs, the vast majority of these were performed in a dilute solution. The interior of the cell is, however, very far from a dilute solution, the typical concentration in the cell can reach as high as 40%. The high concentration of neighboring molecules can considerably influence the behavior, stability and conformation of the molecules. This effect is called macromolecular crowding. Biological processes involving macromolecular structural changes like folding can behave quite differently in such a restricted environment. It has been shown for proteins, that their conformational equilibrium can be shifted due to crowding. E. g. the enzyme phosphoglycerate-kinase is 15 times more active in presence of 20% Ficoll™, because it adopts only the biochemically more active conformation in the crowded environment [14]. The temperature and pressure stability of bovine serum albumin and of lysozyme was affected also considerably by addition of high concentration of macromolecules [15].

In most of the studies in the field of macromolecular crowding, the crowded environment is reached by adding different kind of crowding agents, like dextran, Ficoll™, and PEG (polyethylene glycol).

Only a few studies dealt with the effect of macromolecular crowding on the stability of the GQs. Prof. Sugimoto's lab studied the thrombin binding aptamer (TBA) [16]. They found slight stabilization of TBA in presence of several crowding agents, like EG (ethylene glycol), PEG200 (PEG with average Mw of 200) and PEG4000. These crowding agents caused an increase of the unfolding temperature by 6–8 °C when they were used in 40% at atmospheric pressure. More interestingly they decreased the volume change of the unfolding, by a factor of four.

The effect of high pressure is not limited to understanding of the deep sea life [17,18], but its role as a thermodynamic parameter should not be underestimated as well. Pressure allows accessing volumetric parameters of the systems and volume changes during conformational and also chemical transitions [19]. Such kind of information, which cannot be obtained without pressure experiments, allows deeper understanding of biological processes, and can be relevant even at atmospheric pressure environment.

Only a few pressure experiments have been performed on GQs. Htel was investigated in a limited range of 0.1 to 150 MPa. Most biomolecules (e.g. proteins) are sensitive only to higher pressures; they need typically 400–600 MPa for unfolding [20–23]. This is the first experimental approach which explores the whole available range of the p-T phase diagram up to 1 GPa, where the water starts to freeze at room temperature.

Effect of pressure and of PEG200 on Htel was measured in Sugimoto's lab using UV absorption spectroscopy [24]. They found 6 °C stabilization by 20% PEG200 and a marked pressure destabilization of 0.15 °C/MPa.

Li et al. in Macgregor's laboratory [25] investigated the Htel aptamer and its loop variables up to 160 MPa by absorption spectroscopy. They found a pressure destabilization of the folded GQ structure, the unfolding temperature of Htel decreased by 10 °C in their experimental range.

The effect of deep see osmolyte trimethylamine N-oxide (TMAO) and that of Ficoll™ was investigated in Winter's laboratory [26] in case of Na^+ stabilized human telomeric GQs. They found the Na^+-containing Htel being very pressure sensitive, namely 100 MPa was enough to unfold these quadruplexes at 25 °C. TMAO and Ficoll™ could however save the GQs against pressure and urea induced unfolding. It is known that Na^+ can only weakly stabilize GQs compared to K^+. This might explain the extreme pressure sensitivity of Na^+-Htel.

2. Materials and methods

Htel d(AGGGTTAGGGTTAGGGTTAGGG) and the fluorescently labeled Htel (called as Htel-FRET) containing a FRET pair of FAM and TAMRA were purchased from Sigma. For the infrared measurements D_2O based buffer solutions were used. D_2O was purchased from Cambridge Isotope Laboratories, Inc. Ficoll™ 70 was purchased from GE Healthcare, all other chemicals were purchased from Sigma.

Infrared spectra were measured by a Vertex 80v spectrometer (Bruker, Germany), which was equipped by a diamond anvil cell (Diacell, UK) in order to achieve high pressure. The pressure was measured using the 983 cm^{-1} line of $BaSO_4$ [27]. In some experiments polyethylene foil was used as internal calibrant, which was calibrated previously against $BaSO_4$ (Olah, Somkuti, Smeller unpublished). The experiments were performed by increasing the temperature with a rate of 12 °C/h at constant pressure. In the infrared experiments the Htel aptamer was not labeled fluorescently, unless stated otherwise. The Htel concentration varied from 2 to 75 mg/ml.

Fluorescent spectra were measured by a Fluorolog-FL3 fluorimeter (Horiba Jobin Yvon, France). A homemade reflection mode diamond cell was adopted into the sample holder. The pressure was measured by recording the ruby fluorescence. It was excited by a green HeNe laser (Coherent, USA) and the emitted light was detected by a CCD camera (Andor, UK) attached to a THR1000 monochromator (Jobin Yvon). The temperature was measured in all the experimental setups by a thermocouple connected directly to the high pressure cell. A HH802U type thermometer and the corresponding software from Omega were used to record the temperatures in each 30 s (Omega, USA).

Simultaneous measurement of the fluorescent signal and of the infrared spectrum was performed in a CaF_2 liquid cell. A special optical setup was built for this purpose in the sample chamber of the FTIR spectrometer. Excitation was achieved by focusing the light of a blue power LED (3 W, 600 mA, Digitalsat, Hungary) on the IR cell. The emitted light was transferred to the spectrometer using an optical fiber.

The transition temperature was determined by fitting the temperature dependence of spectral parameters by the following sigmoidal function [28]:

$$y(T) = a + bT + \frac{\Delta a + \Delta bT}{1 + \exp\left(\frac{\Delta H}{R}\left(\frac{1}{T} - \frac{1}{T_m}\right)\right)}$$

Here y is the physical parameter to be fitted (e.g. fluorescence intensity, area of a certain infrared spectral line) a and b are the parameters describing the linear dependence of $y(T)$ below the transition, Δa and Δb are the changes of a and b during the transition, T_m is the transition midpoint, all the other symbols have their usual meaning.

3. Results and discussion

Fig. 2a shows the fluorescence emission spectra of 2 μM Htel-FRET in 100 mM K-phosphate buffer (pH 7.4) at ambient pressure at different temperatures from 25 °C up to 90 °C. One can see the disappearance of the acceptor peak at 580 nm and a simultaneous increase of the donor (FAM) intensity at 520 nm during the heating of the sample. Fitting the donor/acceptor intensity ratio gave a sigmoid curve with the middle point of 62 °C (Fig. 2b). This corresponds to the unfolding temperature of Htel measured by other methods [7,25].

The ratio of the acceptor and donor intensities (A/D) at 25 °C is around 2.5, which is considerably smaller than that of we obtained earlier in case of the thrombin binding aptamer (TBA). TBA has an intramolecular antiparallel (basket) structure, which means, the two chromophores are at the same side of the folded GQ. Low A/D values in case of Htel suggest the predominance of the 3 + 1 hybrid and a very low amount of 2 + 2 basket type conformation.

Fig. 3a shows the infrared spectrum of Htel in D_2O at pD 7.4 (0.2 M Bis-Tris, 0.2 M KCl, 0.2 mM EDTA). Above 2000 cm^{-1} one can see the wide absorption bands of H_2O, D_2O and diamond. The CH region is also clearly seen at 2800–2900 cm^{-1}. Sharp peaks of polyethylene (used for pressure determination) appear around 730 cm^{-1}. The absorption bands sensitive for the structure of the GQ are however located in the region of 800–1750 cm^{-1}. This is enlarged in Fig. 3b and c.

The most intense band between 1665 and 1689 cm^{-1} is characteristic of the $C_6{=}O_6$ carbonyl stretching vibration of guanine bases. It can be found around 1665 cm^{-1} in case of free guanine, near 1672 cm^{-1} in case of Hoogsteen type G-G pairs and shifted to 1689 cm^{-1} in the presence of Watson-Crick G-C base pairs [29,30]. During heating this peak shifted from 1676 cm^{-1} to 1667 cm^{-1} referring to the disruption of hydrogen bonds. We plot the ratio of the absorbance at these frequencies (Fig. 4a) which follows a sigmoid curve as the function of temperature indicating the unfolding of the G quadruplex. The shoulder of this band at 1630 cm^{-1} is assigned to thymine ring vibration, it did not change considerably [31].

The band around 1586 cm^{-1} refers to the ring C=C vibration, its shoulder at 1568 cm^{-1} is assigned to C=N and C−N stretching not including the N_7 atom [29,32]. Upon heating it is shifted to 1575 cm^{-1} and the absorbance is increased. From the ratio of the absorbance values we can also follow the unfolding process and determine the melting temperature (Fig. 4b).

The most characteristic band of G quadruplexes is located at 1537 cm^{-1}. This involves the C=N and C−N stretching with a major contribution from the N_7 atom, it was observed only in the presence of G-G Hoogsteen type base pairs [29]. The peak disappeared at higher temperature indicating the unfolding of the quadruplex (Fig. 4c) and reappeared at 1525 cm^{-1} after cooling down the sample to room temperature. This shift can be explained by a H/D exchange. Originally these H atoms in the quadruplex were not accessible for the deuterated solvent, however, after unfolding they became accessible and exchanged. In a second heating cycle the peak at 1525 cm^{-1} disappeared at high temperature and reappeared again after cooling without any further shift. There were no differences in the melting temperature of the two heating cycles. The unfolding process was reversible except of the H/D exchange.

The band around 1090–1077 cm^{-1} is characteristic of the symmetric stretching of phosphate groups. Its position is at 1090 cm^{-1} when the guanine nucleotide is involved in a tetraplex, and at 1077 cm^{-1} if the nucleotide base is in the loop and not paired [32]. During melting the ratio of the two peaks is changing since 1090 cm^{-1} is decreasing and 1077 cm^{-1} is increasing and the unfolding can be followed (Fig. 4d).

The positions at 865 cm^{-1} and 810 cm^{-1} are characteristic of N type (C3' endo), the band at 836 cm^{-1} for S type (C2' endo) sugar conformations [33]. In this region we could observe a peak only at 836 cm^{-1} which suggests that sugars in Htel quadruplex adopt S type geometry. This peak was also found by Guzman et al., in case of several GQ forms [34]. It is also known from the literature, that folded GQs show S type (C2' endo) sugar geometry [35].

All the spectral changes of the infrared spectrum could be fitted with a sigmoid curve. The transition temperatures were slightly lower (e.g. 80.3 °C and 81.1 °C at 300 MPa and 75 mg/ml) for the absorption lines which represent vibrations near or at the sugar-phosphate backbone (1584 cm^{-1} and 1090/1070 cm^{-1} respectively). The bands at 1676/1667, 1537 and 1526 cm^{-1} represent vibrations inside of the GQ, they change at slightly higher temperatures (83.5 °C). This suggests that water is penetrating into the folded structure from the outside, and the internal Hoogsteen hydrogen bonds disrupt only as the last step of the unfolding.

Looking to the transition temperatures at ambient pressure, we noticed a significant difference between the infrared and fluorescence measurements. The transition temperatures measured in the infrared experiments are systematically higher than the one obtained from the fluorescence spectroscopy. The difference is about 7–8 °C depending on the concentration.

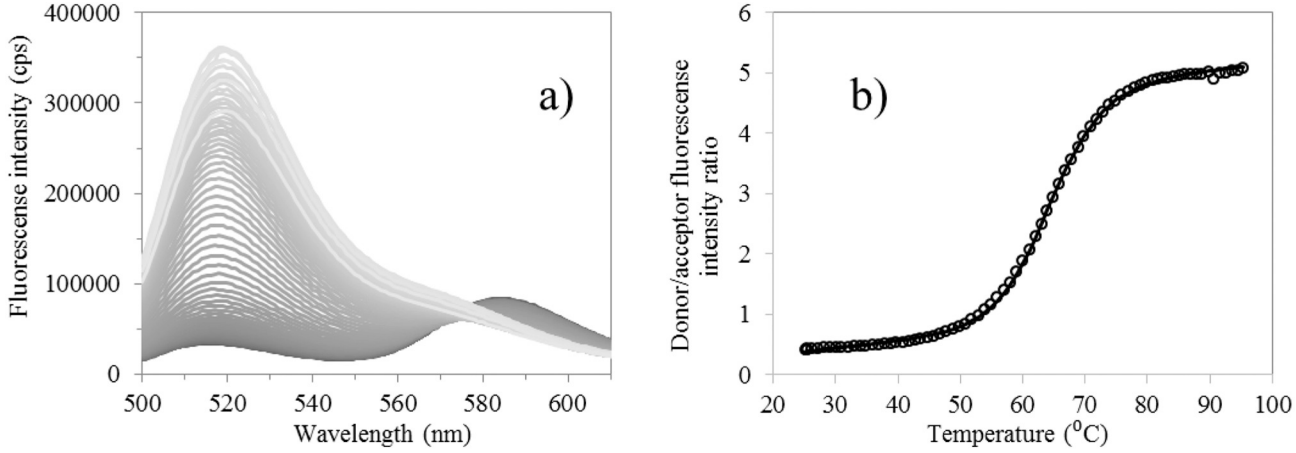

Fig. 2. a) Fluorescence emission spectra of 2 μM Htel-FRET in 100 mM K-phosphate buffer (pH 7.4) at ambient pressure at temperatures from 20 °C (dark line) to 90 °C (pale line). b) The ratio of donor/acceptor fluorescence emission intensity vs temperature. The experimental points are fitted with a sigmoid curve presented in the Materials and methods section.

Risitano [36] observed a stability increase of 0.5 K by increasing the concentration from 0.1 to 1 μM. Although the transition temperature of quadruplexes is known to be slightly concentration dependent we wanted to rule out the effect of other factors like presence of fluorophores on the stability of the GQs.

In order to exclude the effect of the fluorescence labeling on the stability of the folded GQ structure, we decided to measure both spectra simultaneously on the same Htel-FRET sample. A special optical setup was constructed for this reason into the sample compartment of the FTIR spectrometer. The simultaneous experiments showed the same difference between the transition midpoints as it was in case of separate fluorescence and FTIR measurements (data not shown). This suggests that the unfolding of the GQ structure is not a single step process, there are intermediate conformations appearing during the unfolding. FRET

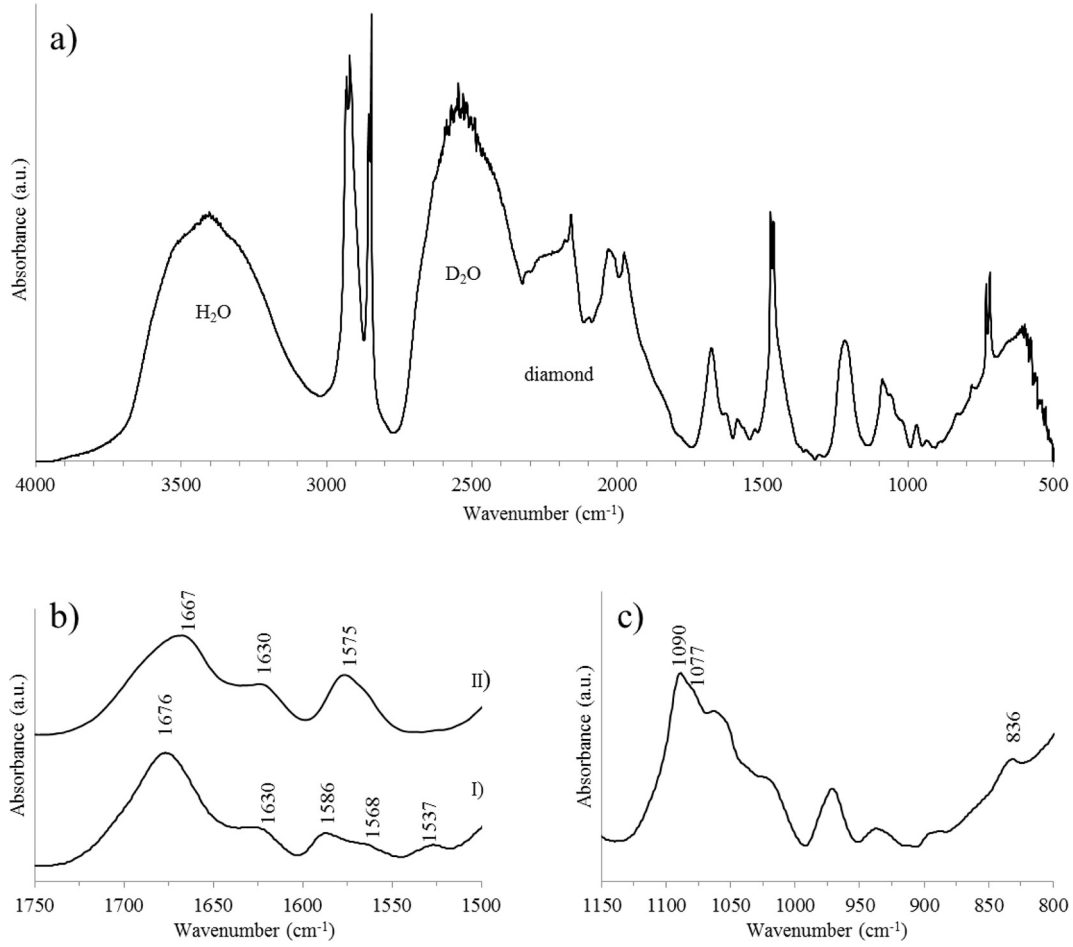

Fig. 3. a) Infrared spectrum of 75 mg/ml Htel in D_2O at pD 7.4 at 300 MPa. b) The infrared region containing the vibrational bands sensitive to the conformation of the DNA. Curves I and II were measured before and after the first heating cycle. c) The infrared region containing the vibrational bands sensitive to phosphate and sugar conformation.

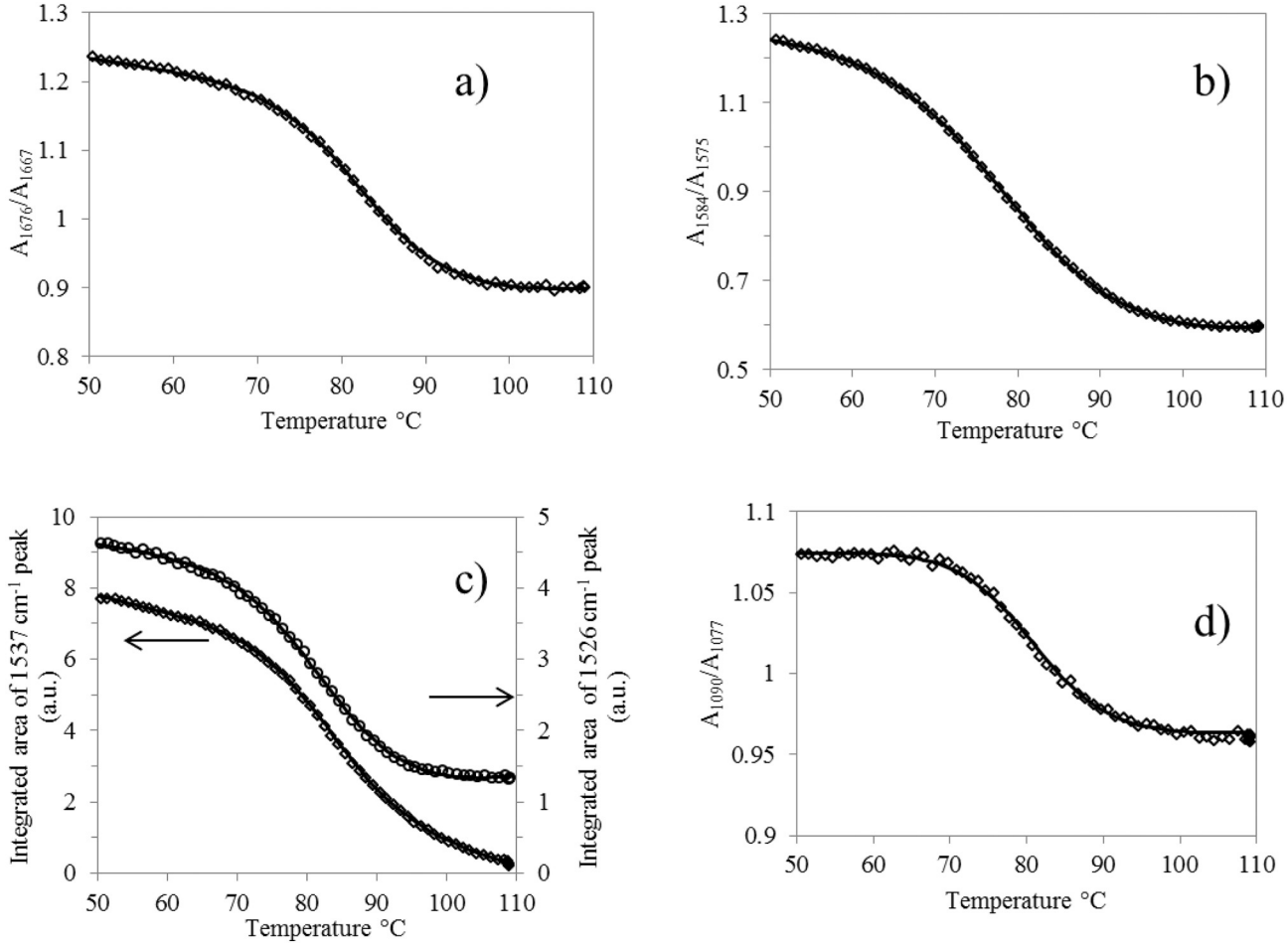

Fig. 4. Temperature dependencies of the most characteristic infrared vibrations of Htel in D_2O at pD 7.4 at 300 MPa pressure: a) absorbance ratio of 1676 and 1667 cm^{-1} bands b) absorbance ratio of 1584 and 1575 cm^{-1} bands c) integrated area of the 1537 and 1526 cm^{-1} bands measured during the first and second heating cycles respectively d) absorbance ratio of the 1090 cm^{-1} and 1077 cm^{-1} bands (experimental conditions are the same as in Fig. 3).

and IR spectroscopies are sensitive to different conformational steps at this process. As we mentioned above concluding from the FTIR experiments, the first changes of the unfolding happen in the outer part of the molecule. This might be the reason for the earlier onset of the fluorescence signal changes during the heating. Zhang and Balasubramanian [37] found a transition state while studying the folding kinetics of Htel. It is important to note also that the Htel GQ structure is stabilized by two K^+ ions [9,7,8], which can also explain the deviation from the two-state transition.

Fig. 5 shows the pressure-temperature phase diagram of Htel without crowding and at different self-crowding conditions. As we can see the folded GQ state is destabilized by pressure at low concentrations. The pressure dependence of the transition temperature can be fitted by a linear function with the slope of −28.5 MPa/°C. Similar trend was observed by Takahashi [16] and MacGrgor [25] using UV absorption spectroscopy, although their slope differs slightly from our one. From the fit one could determine the ΔH of the transition, which was (180 ± 20) kJ/mol. This is in agreement with other studies at atmospheric pressure [24]. We obtained a ΔV value of - (19 ± 3) cm^3/mol for the unfolding using the Clausius-Clapeyron equation. This corresponds roughly to the volume of one water molecule. It was pointed out, that the hydrational state change of the cations upon unfolding of the GQ plays an important role in the volume change of the system [25].

Self-crowding condition alone can stabilize the GQ form, as it can be seen from the higher transition temperatures at atmospheric pressure. Applying pressure, the folded GQ structure will be stabilized further.

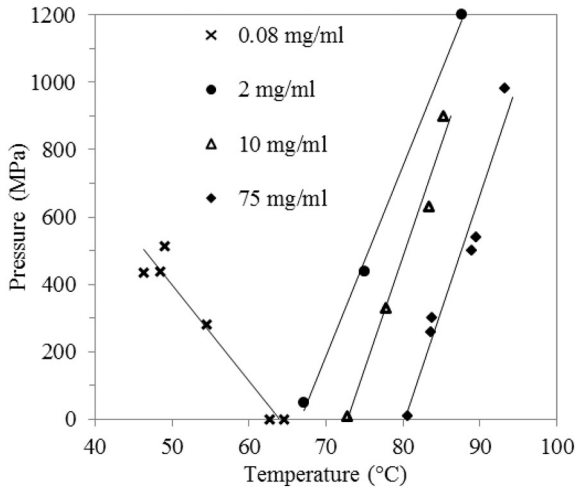

Fig. 5. The pressure-temperature phase diagram of Htel at different self-crowding conditions.

This means, that under self-crowding conditions we obtained the reversal of the trend observed in case of dilute solutions. The slope dT_m/dp of the transition line on the phase diagram is positive in the crowded samples. The dT_m/dp, ΔH and ΔV values obtained from the fitting are summarized in Table 1. The ΔV value is in the range of 6–7 cm^3/mol in case of high concentrations. The ΔV and the enthalpy change values

Table 1
fitted thermodynamical parameters of unfolding of Htel at different concentrations.

Htel concentration (mg/ml)	0.08	2	10	75
dp/dT (MPa/°C)	−28.5	56.2	67	68.4
ΔH (kJ/mol)	180 ± 20	147 ± 20	158 ± 15	158 ± 20
ΔV (cm^3/mol)	−19 ± 3	7.4 ± 1	6.6 ± 1	6.4 ± 1

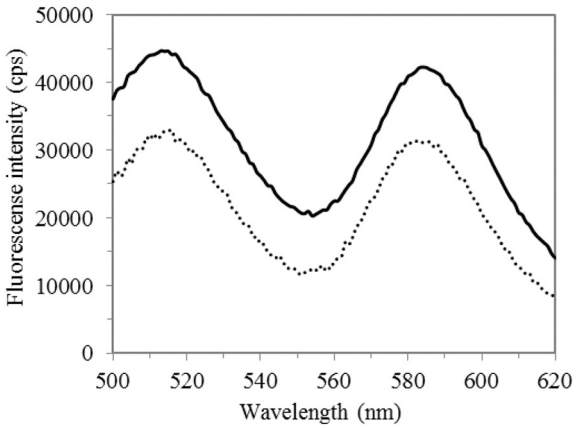

Fig. 6. Fluorescence spectra of Htel sample containing small amount of Htel-FRET: 10 μM (0.08 mg/ml) Htel-FRET + 10 mg/ml Htel (not labeled) at ambient pressure at 30 °C in 100 mM K-phosphate buffer (pH 7.4).

depend only slightly on the concentration in the range of 2–75 mg/ml.

One can argue, that the structure formed under high concentration might be different from the one formed in the dilute solution. A natural assumption would be to suppose the presence of intermolecular GQ structures at high concentration, which are composed of four separate strands. (Note that the normal GQ is formed by a single oligomer!) In order to rule out this case we prepared a sample by mixing 10 mg/ml (1.4 mM) fluorescently not labeled Htel with 10 μM Htel labeled by our FRET pair. If an intermolecular tetramer GQ was formed, the strands would be practically straight even in the folded case, therefore the FRET efficiency should have been decreased considerably already at low temperature, where the GQ form is stable. As it can be seen in Fig. 6 this is not the case. Therefore, we can rule out the formation of intermolecular GQs at high concentration.

Another argument for the monomolecular structure at high concentration is the similarly high concentration used in the NMR studies [7,9]. These experiments provided the 3D picture of the monomolecular GQ structures adopted by Htel.

The effect of high concentration of DNA aptamers was studied by the Vorlickova and McGregor groups. The former group performed Raman spectroscopic study on the $dG_3(TTAG_3)_3$ aptamer [35]. This is similar to Htel, but the first adenine base of Htel is missing. Increase of the DNA concentration clearly increased the intensity of the second spectral component provided by the singular value decomposition analysis. This fact was interpreted as a shift in the equilibrium between two conformers present in the solution. The spectral changes suggest increased amount of *anti* conformation of the guanine bases at higher concentration, which is consistent with the predominance of the parallel quadruplex conformation relative to the hybrid one, where the *syn* and *anti* conformers are present roughly in the same amount. Palacky et al. did not quantify the relative amount of these conformers as function of the concentration. The appearance of the parallel quadruplex form was, however, observed only after a heating cycle, which changed also the viscosity of their sample. This questions the monomolecular nature of the DNA oligomers in their experiments. In our case however the spectra showed identical features during the first and second heating cycles (apart from the H/D exchange discussed before). This observation allows us to argue again for the absence of intermolecular GQ structures in our sample.

McGregor's group performed [38,39] a systematic study on the concentration dependence of different oligodeoxyribonucleotides including Htel, using circular dichroism (CD) spectroscopy. They interpreted the spectral changes by increase of the parallel GQ structure at high salt concentrations. A very slow conformational change with a characteristic time in the order of magnitude of few hours was also observed, and assigned to the aggregation of the monomolecular GQs. However, the latter observation was performed in presence of Na^+ not K^+. Sodium stabilized GQs are known to be less stable compared to potassium stabilized ones, which can explain their different aggregation propensity.

Our results, showing an increased unfolding temperature at higher Htel concentration, are in agreement with the above mentioned experimental findings [35,38,39]. The formation of parallel structures can also be understood from the absence of the 920 cm^{-1} band in the infrared spectrum, the one which is characteristic for existence of guanosines in *syn* conformation [34].

We would, however, rule out the aggregation of Htel monomers in our high pressure experiments. Pressure is known to counteract the association of monomers. This was clearly demonstrated in case of several proteins, where the aggregates and oligomers were sensitive to pressure. Even pressures, which were unable to distort the internal structure of the protein, were able to dissociate their associates [20,22,40,41].

It has to be mentioned, that the first 3D structure determined by x-ray crystallography shows also a parallel structure [42]. Latter NMR studies found 3 + 1 hybrid structures, and the crystal structure was attributed to the high concentration used for crystallization [35]. The proposed propeller type parallel structure contains big loops with thymine and adenine bases outside of the molecule. This structure might also contribute to the pressure stability of the high concentration form of folded Htel.

Our most interesting finding is that the volume change associated to the unfolding of the GQ structure is reversed under self-crowding conditions. While in dilute samples the ΔV is negative, it turns into a smaller but positive value in crowded environment. This has consequences for the pressure behavior, namely the negative ΔV leads to pressure destabilization, while in case of positive unfolding volume change, pressure stabilizes the folded state.

There are two possible explanations for the sign change. First it could be explained by molecular mechanism of the unfolding. It was pointed out, that unwinding of the double stranded DNA is quite pressure insensitive, the ΔV is near zero or small positive (< 5 cm^3/mol) [43,44]. The reason for the pressure sensitivity of such exotic DNA forms like the GQ lies in the presence of the stabilizing central ions. These are present in the folded GQ without their hydration shell. During the unfolding GQ will release the ion which adopts water molecules to build its hydration shell. Since the hydration shell has higher density, volume decrease is measured [25]. The hydration ΔV overcompensates the small ΔV associated to the unfolding of the nucleic acid itself. In crowded environment, however, the hydration shells of the GQ-s can occupy significant part of the solvent making it more ordered, therefore the volume gain caused by the hydration of the released central ions is much lower. This results a small positive ΔV. This effect could be relevant in case of 75 mg/ml, where the calculated average distance of the DNA oligomers is 5 nm, comparable with the diameter of the folded GQs (3–4 nm calculated from the PDB data).

The second explanation for the sign change of ΔV can be based on the fact, that appearance of parallel conformations of Htel were suggested at high concentration. In this parallel Htel form the bases in the loops are completely exposed to the solvent. This could also reduce the volumetric gain of the unfolding, compared to the case, where the loops are less solvent exposed. In the latter case the unfolding leads to increased hydration shell, decreasing the volume of the system containing

the unfolded oligomer.

Either of the above mechanisms is dominant in the stabilization of the GQ structure of Htel, these observed volumetric aspects could be relevant in biological systems, since the concentration of macromolecules in the cell nucleus can reach a value of 400 mg/ml [45].

4. Conclusion

Our results show a marked effect of the crowding condition on the stability of the Htel aptamer. Under self-crowding conditions, the volume change of the unfolding will turn from negative to positive, which means, the unfolded state will have higher volume than the folded GQ, in highly self-crowded environment. This could have important consequences, taking into account, that the DNA concentration in the cell nucleus is quite high, can reach or even exceed the one we used in the FTIR experiments. The effect of the self-crowding environment on the unfolding volume can be explained by the different hydration volume changes in the dilute and crowded environments.

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