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The effect of mono- and divalent cations on *Tetrahymena thermophila* telomeric repeat fragment. A photon correlation spectroscopy study

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The structure of the *Tetrahymena thermophila* telomeric sequence d(TGGGGT)$_4$ was studied by photon correlation spectroscopy (PCS) in aqueous solution in the presence of NaCl, KCl and $SrCl_2$. The sample studied was polydisperse in all conditions studied. Translational diffusion coefficients D_T describing the diffusion modes observed were determined. On the basis of a comparison between the experimental D_T values with those calculated assuming the bead model, two forms were identified as telomeric quadruplex structures: monomer and tetramer. In the presence of $SrCl_2$ formation of aggregates was observed, with a size that reached several micrometres. The relative weighted concentrations of the structures observed for different concentrations of a salt and DNA were determined. The results obtained in the presence of monovalent ions were qualitatively similar and could be presented in a coherent plot in which the concentration of salt was expressed by the number of ions per DNA molecule. A large number of ions per DNA molecule favoured tetramer formation while a small number favoured the monomer form. A structural phase transition from the monomer to the tetramer induced by a change in the number of ions per DNA molecule was observed. The main difference between the results for Na^+ and K^+ was a greater effectiveness of the K^+ ions in formation of tetramers. The effect of Sr^{2+} ions on the structures formed was different than that of the monovalent ions. The results obtained in the presence of Sr^{2+} could not be described as a function of the number of ions per DNA molecule.

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Abbreviation: PCS, photon correlation spectroscopy.

Telomeric DNA (Blackburn, 1991; Biessmann & Mason, 1994; Makarov *et al.*, 1997; Greider, 1999; Griffith *et al.*, 1999; McCormick-Graham *et al.*, 1997; Liu *et al.*, 1999) has been a subject of intense study showing that *in vitro* single G strands can interact either with themselves or with other G strands to form different four-stranded structures known as quadruplexes. The structures formed by a single G strand appear to have large effects on the biochemical properties of telomeres (Greider & Blackburn, 1985; Linger *et al.*, 1995; Blackburn, 1999; Neidle & Read, 2001). The quadruplex structures are formed owing to hydrogen bonds forming so-called G quartets. Depending on the sequence, DNA concentration, type and concentration of ions and sample preparation, the structures formed differ in the strand stoichiometry (monomer, dimer, tetramer), strand polarity (parallel *versus* antiparallel), glycosidic torsion angle variation and loop geometry (Keniry, 2001; Williamson, 1994; Lee, 1990). It has been observed that some ions prefer formation of particular structures (Keniry, 2001), that can then lead to structural phase transitions induced by a change in the ion concentration. Such transitions occur in systems with one type of cations present only, e.g. with K^+ cations (Miura & Thomas, 1994; Miura *et al.*, 1995) or in systems with a few types of cations (Miura & Thomas, 1994; Miura *et al.*, 1995; Sen & Gilbert, 1990). The presence of the ions favours formation of certain structures while a high concentration of DNA shifts the equilibrium towards formation of structures made of many strands (Sen & Gilbert, 1992; Williamson, 1994; Miura & Thomas, 1994). Also the method of sample preparation can influence the equilibrium of the G-quadruplex forms (Chen, 1992). The first study of the *Tetrachymena* $d(G_4T_2)_4$ sequence by gel electrophoresis in non-denaturing polyacrylamide gels (Henderson, 1987) showed a high structural polymorphism of this sequence. The presence of compact forms with enhanced electrophoretic mobility relative to that of a single unfolded strand was demonstrated. An NMR study performed by the same authors proved the presence of G–G bonds as well as of several guanosines in *syn* conformation. Williamson *et al.* (1989) studied the sequence in the presence of the alkali ions: Na^+, K^+, Rb^+, Li^+ and Cs^+. In the presence of Na^+, K^+, Cs^+ formation of a compact structure of enhanced electrophoretic mobility was observed, and in the presence of K^+ additional formation of several structures with lower mobility than that of a single strand was observed. On the basis of the results obtained by various methods (i.e. gel electrophoresis, methylation protection and interference, and UV cross-linking), those authors proposed a structural model of the compact form as a monomeric quadruplex. Finally, the structure of the compact form in the presence of Na^+ was solved using a combined NMR-molecular dynamics approach (Wang & Patel, 1994). That study confirmed that the compact form is a monomeric quadruplex containing three stacked G-quartets connected by three linker loops, but with a different folding topology to that proposed by Williamson *et al.* (1989). The effect of Na^+ and K^+ ions on the structures of the sequence $d(G_4T_2)_4$ was also studied by gel electrophoresis, imino proton NMR and circular dichroism (Hardin *et al.*, 1991). The authors proposed a model of quadruplexes in which the hairpins and linear dimers, resulting from interaction between guanines, are intermediates of the final structures of monomers, dimers and tetramers. In the presence of sodium, the formation of monomers, hairpins, dimers and to a small degree of tetramers, was observed, while the presence of potassium significantly enhanced the formation of tetramers and intermediate forms. Similar results were obtained by Chen (1992) on the basis of CD spectroscopy and gel electrophoresis in non-denaturing conditions in the presence of Na^+, K^+ or Sr^{2+}. In such conditions the sequence $d(G_4T_2)_4$ formed many struc-

tures that were often dependent on sample history, of particular importance was the temperature of preincubation. The presence of tetramers was observed in all conditions studied. Moreover, a number of structures intermediate between dimer and tetramer were observed, that showed different electrophoretic mobility. The presence of 5 mM of strontium significantly enhanced the formation of tetramers and reduced the concentration of intermediate forms. A similar effect of an increasing concentration of tetramers at the expense of intermediate forms was also observed in the presence of potassium, but the effect of Sr^{2+} was even greater.

The same sequence but of a length of ten nucleotides – $d(G_4T_2G_4)$ was studied by atomic force microscopy (Marsh *et al.*, 1995), where the authors showed that this sequence can form large, self-assembling nanostructures, called G-wires, in Na^+/Mg^{2+} and K^+/Mg^{2+} containing buffers. The length and the height of the G-wires were determined by the metal ions present during the self-assembly process.

In the present study photon correlation spectroscopy (PCS) was applied to identify the types of structures formed by the sequence $d(G_4T_2)_4$ in the presence of Na^+, K^+ and Sr^{2+}. In particular, the effect of the concentrations of the ions and DNA on the structural equilibrium was analysed. A number of conformational phase transitions were observed. The PCS method is particularly suitable for such studies as it is non-invasive and permits analysis of global ordering in solution.

MATERIALS AND METHODS

$d(5'\text{-TGGGGT-}3')_4$ was synthesised by the Midland Certified Reagent Company (Midland, U.S.A.) using the cyanoethyl-phosphoramide method. The sample was purified by HPLC and was 90–95% pure. For PCS measurement, samples were dissolved in 10 mM Tris/HCl, pH 7.3, and final salt concentrations were adjusted by dialysis. The dialysed samples were filtered through a 0.22 μm pore cellulose Millipore filter. In PCS experiments the light source was an Ar^+ laser operating at λ = 488 nm with an output power of 480 mW. The vertically polarised component of scattered light was analysed by an ALV-5000 digital correlator. The correlation functions were measured at 20°C for the scattering angle of θ = 90°. The correlation function was analysed using a multiexponential model, and gave the relaxation times τ_i describing the processes of translational diffusion and the amplitudes A_i of these processes describing their contributions in total light scattering. The uncertainties of measurements were estimated within about 5.5% of the determined values. Translational diffusion coefficients were calculated from the formula $D_T = \frac{1}{\tau q^2}$, where q is the scattering vector defined by the expression $q = (4\pi n/\lambda)\sin(\theta/2)$, where n is refractive index of the solution, λ is the wavelength of the incident light, and θ is the observation angle of the scattered light relative to the transmitted beam. Analysis of the correlation function also provided information on the relative weight concentrations of the structures taking part in the scattering. The amplitude of the diffusion mode A is proportional to the intensity of the light scattered by a structure corresponding to a given diffusion mode I_s. The intensity of the scattered light I_s is also proportional to the product cM, where M is the molecular mass and c the concentration of the sample. When there are n structures contributing to the scattering the relative weighted concentration of the i–th structure $\tilde{c}_i$ can be calculated from the dependence:

$$\tilde{c}_i = \frac{c_i}{c_{tot}} = \frac{\frac{A_i}{M_i}}{\sum_{j=1}^{n} \frac{A_i}{M_i}}$$

where $c_{tot} = \sum_{j=1}^{n} c_j$ is the total concentration of DNA in the sample. When the mass of the scattering object was unknown, it was approximated as proportional to σ^3, where σ is the hydrodynamic radius of a given structure related to the diffusion coefficient through the Stokes-Einstein formula $D_T = kT/6\pi\eta\sigma$, where k is the Boltzmann constant, T is temperature and η is the solvent viscosity.

RESULTS AND DISCUSSION

Hydrodynamic modelling

In this study the possibility of occurrence of the following telomeric quadruplex structures in the sample was considered: monomer, dimer and tetramer (Figs. 1a, b, c). Theoretical values of the translational diffusion coefficients characterising these structures were calculated on the basis of the bead model (Banachowicz *et al.*, 2000). According to the idea of the bead model, each nucleotide was divided into two groups of atoms: the first containing the nitrogen base and the second the sugar and phosphate residue. Each of these groups was replaced by a bead of a radius σ = 5 Å, positioned at the geometric centre of a given group. For the obtained system of beads the translational diffusion coefficient was numerically calculated using the algorithm proposed by Richard Pastor, taking into account the Rotne–Prager tensor of hydrodynamic interactions for partly overlapping elements (Rotne & Prager, 1969).

The calculations for the monomer were performed for the coordinates taken from the Brookhaven Protein Data Bank. The structure of the sequence studied $d(TGGGGT)_4$ was obtained on the basis of the structure of the monomer of $d(TTGGGG)_4$ resolved by NMR, access code 186d (Wang & Patel, 1994).

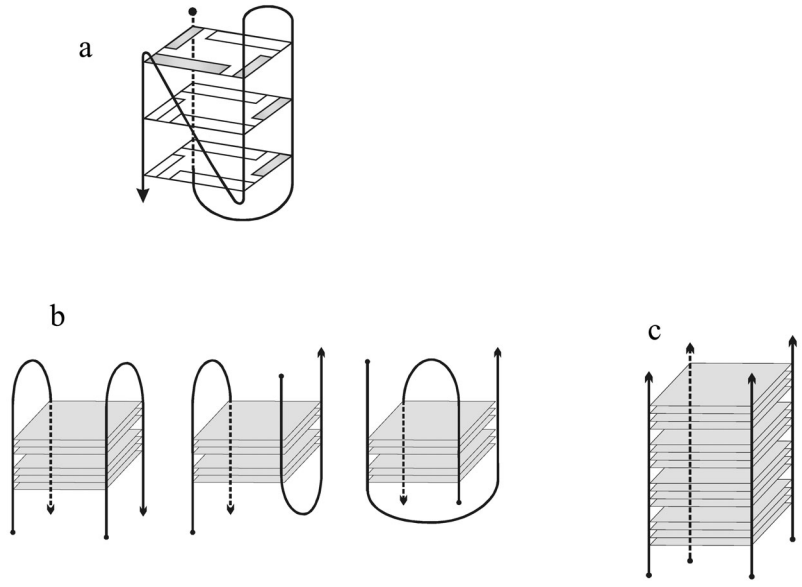

Figure 1. Schematic drawing of the quadruplexes structures that can be adopted by telomeric G-strand sequence $d(T_2G_4)_4$.

a. Monomer (Wang & Patel, 1994). Solid and dashed lines represent the sugar phosphate backbone. Solid circle indicates the 5′ end of the strand and arrow the 3′ end. Bases indicated with shaded rectangles are in the *syn* glycoside bond conformation while those with unshaded rectangles are in the *anti* conformation. **b**. Dimers considered in the study. **c**. Tetramer. Shaded squares in **b** and **c** represent guanine quartets.

The structure of the $d(TTGGGG)_4$ monomer was modified by removing one thymine from the 5′ end and attaching it to the 3′ end. The

modification was performed with the help of the program HyperChem6, which was also used for construction of dimers and tetramers of the sequence studied. The translational diffusion coefficient calculated for the monomer is $D_T = 1.42 \times 10^{-6}$ cm^2/s, while for the tetramer it is $D_T = 0.86 \times 10^{-6}$ cm^2/s. Dimer structures with parallel, antiparallel and diagonal loops were considered and the translational diffusion coefficients calculated for them were similar and close to $D_T = 1.2 \times 10^{-6}$ cm^2/s.

Photon correlation spectroscopy

Coefficients of translational diffusion for the structures formed by d(TGGGGT)$_4$ in the presence of Na^+, K^+ and Sr^{2+} ions in different concentrations were determined by photon correlation spectroscopy (PCS). The sample was polydisperse under all conditions studied. Analysis of the first 60 channels of the correlation function was sufficient for covering the whole range of variation of the functions studied.

Results for K^+

The equilibrium concentrations of KCl studied were 0.1 mM, 1 mM and 10 mM. Two diffusion modes were observed in the whole range of concentrations of the salt and DNA. The dependence of the values of D_T corresponding to these modes on the DNA concentration is shown in Fig. 2. The observed D_T dependence on DNA concentration suggests strong intermolecular interactions. Comparisons of the experimental and theoretical D_T values were performed at the limit of zero concentration of DNA approximating the situation with non-interacting particles. For 1 mM and 10 mM KCl, the values of D_T extrapolated to zero DNA concentration tended to the limit value of 1.4×10^{-6} cm^2/s for one mode, and to 0.8×10^{-6} cm^2/s for the other. These values are in agreement with those found theoretically for monomer and tetramer quadruplexes, respectively. We therefore assume that one of the diffusion modes is related to monomers and the other one to tetramers. Formation of tetramers of the sequence studied in the presence of K^+ ions has already been indicated by Hardin *et al.* (1991) and Chen (1992). For low concentrations of KCl equal to 0.1 mM, the extrapolation of the

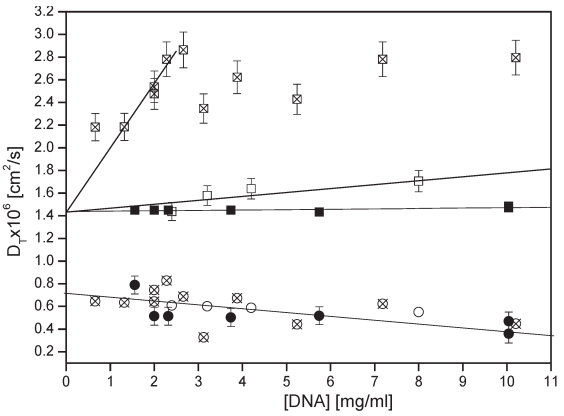

Figure 2. The measured translational diffusion coefficient of the two forms of the telomere *versus* telomere concentration at 0.1, 1 and 10 mM KCl.

Symbols: 0.1 mM KCl ⊠, monomer, ⊗, tetramer; 1 mM KCl □, monomer, ○, tetramer; 10 mM KCl ■, monomer, ●, tetramer.

D_T values related to monomers to zero DNA concentration is difficult because of the low ionic strength of the solution, which means that electrostatic interactions are poorly screened so that even for very small DNA concentrations the molecules can not be assumed as non-interacting. This results in high initial slope of the $D_T(c)$ dependence and a higher error of the extrapolated value.

In order to test the equilibrium between the monomer and tetramer structures, their relative weighted concentrations were determined *versus* the concentrations of the salt and DNA. A typical plot of the relative weight concentrations of the monomer and tetramer *versus* the KCl concentration for the DNA

concentration of 1.6 mg/ml is shown in Fig. 3a. At low salt concentration, the monomeric quadruplex dominates and forms about 80% of the whole DNA weight concentration in the sample. With increasing concentration

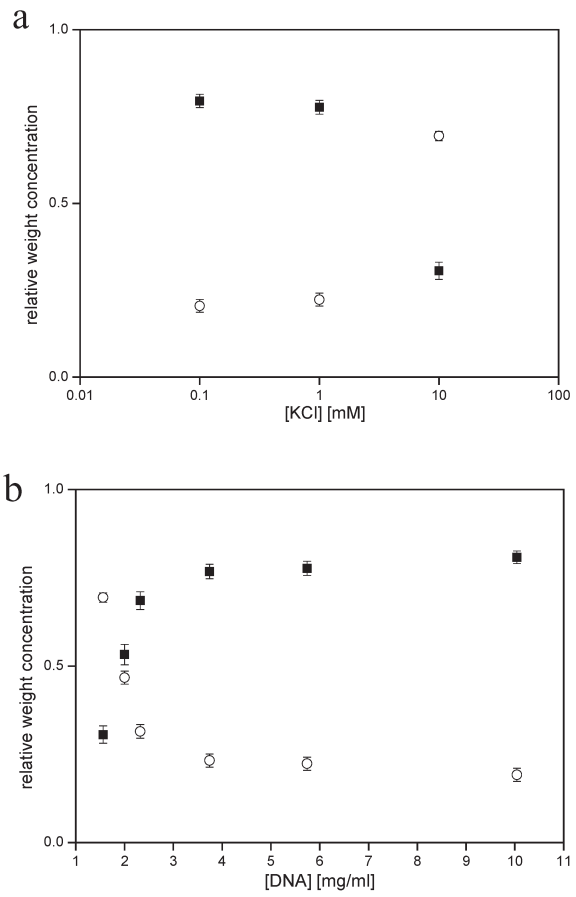

Figure 3. Relative weight concentrations of the monomer and tetramer in the presence of KCl.

a. Relative weight concentrations of the monomer ■ and tetramer ○ *versus* KCl concentration. Telomere concentration is constant and equals 1.6 mg/ml. **b**. Relative weight concentrations of the monomer ■ and tetramer ○ *versus* DNA concentration at 10 mM KCl.

of KCl, the weight concentration of the tetramer increases at the expense of the monomer. For KCl concentrations in the range between 1 and 10 mM a structural transition is observed. For 10 mM KCl the weight concentration of the tetramer makes about 70% of the whole DNA in the sample. A typical dependence of the relative weight concentrations of the monomer and tetramer on DNA concentration, for 10 mM KCl is shown in Fig. 3b. As indicated, at higher DNA concentrations the monomer is dominant. With decreasing concentration, formation of the DNA tetramer at the expense of the monomer is observed, so that at a DNA concentration close to 2 mg/ml the relative weight concentrations of the two forms are the same. In low concentrations of DNA, the tetramer is dominant. This is untypical because usually higher DNA concentrations favour formation of multi-stranded structures (Sen & Gilbert, 1992; Williamson, 1994; Miura & Thomas, 1994). The results shown in Fig. 3a and 3b suggest that the best way to describe the equilibrium between the monomer and tetramer is by expressing the salt concentration in terms of the number of ions per DNA molecule. The plot of the relative weight DNA concentration *versus* the number of ions per DNA molecule is shown in Fig. 4. It was made on the basis of the data from all series of measurements for different

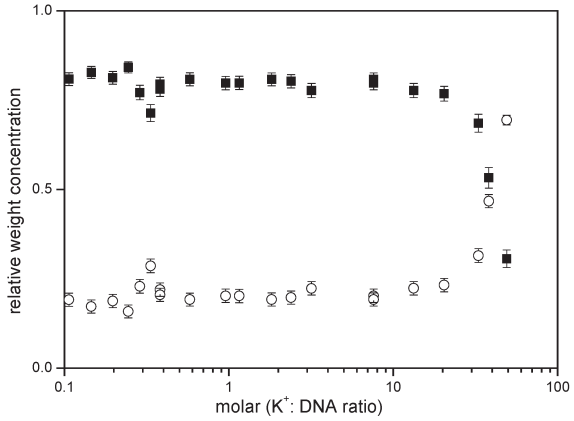

Figure 4. Relative weight concentration of the monomer ■ and tetramer ○ as a function of KCl concentration.

KCl concentration is presented as the number of K^+ ions per DNA molecule.

concentrations of the salt and DNA. The results are arranged along two curves. For a small number of ions per DNA molecule the monomeric quadruplex dominates in the solution, for about 40 K^+ ions per DNA molecule the weight concentrations of the monomer and tetramer are the same, and for a higher

number of ions per DNA molecules the linear tetramer dominates.

Results for Na^+

The measurements for NaCl were performed for its equilibrium concentrations of 10 mM, 100 mM, 150 mM, 300 mM and 500 mM. The results obtained for this salt have been presented elsewhere (Włodarczyk *et al.*, 1999), so only a brief discussion is included here. Similarly to KCl, two diffusion modes were observed in the whole range of NaCl and DNA concentrations studied. By extrapolating the D_T value to zero DNA concentration, the structures related to the modes were identified as monomer and tetramer quadruplexes. Because the NaCl concentrations studied were much higher than those for KCl, it was also possible to study the behaviour of D_T at the limit of high salt concentration, when the electrostatic interactions were strongly screened, and so the D_T should tend to values calculated for non-interacting particles. Figure 5 shows D_T values *versus* NaCl concentration: the D_T values for the monomer mode indeed tend to the theoretically found value for non-interacting monomer quadruplexes which equals 1.42×10^{-6} cm^2/s. The extrapolated value of D_T for the tetramer mode tends to 0.80×10^{-6} cm^2/s which is also in good agreement with the calculated value of D_T equalling 0.86×10^{-6} cm^2/s. The relative weight concentrations of the monomer and tetramer structures were determined *versus* the concentrations of the salt and DNA. As for KCl, all results obtained in the presence of NaCl can be presented in a single plot when the concentrations of the salt and of DNA are expressed in terms of the number of sodium ions per DNA molecule. The relevant plot is shown in Fig. 6. As follows from the plot there is a structural phase transition at about 100 Na^+ ions per DNA molecule, a value that is approximately twice as large as for K^+ ions. For the number of Na^+ ions per DNA molecule smaller than 100, monomer dominates, while the tetramer is dominant at higher molar ratios. Above the value of 6000 Na^+ ions per DNA molecule, the entire DNA within the sample transforms into the tetramer.

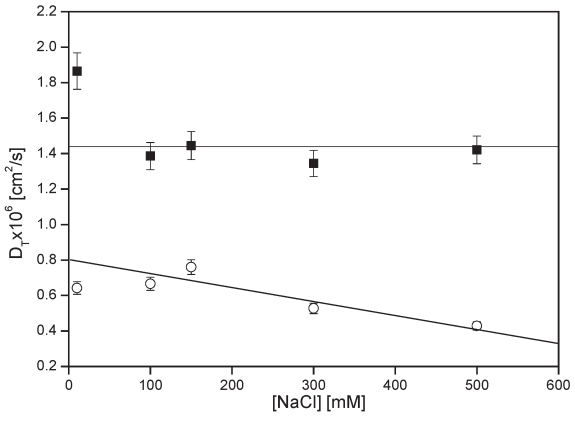

Figure 5. The measured translational diffusion coefficient of the two conformations of the telomere *versus* NaCl concentration at a constant telomere concentration of 20 mg/ml.

Monomer ■, tetramer ○.

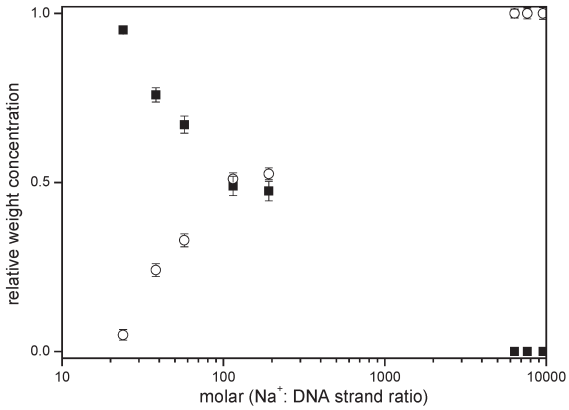

Figure 6. Relative weight concentration of the monomer ■ and tetramer ○ as a function of NaCl concentration.

NaCl concentration is presented as the number of Na^+ ions per DNA molecule.

In general, the effect of Na^+ ions on the formation of quadruplex structures is similar to that of K^+ ions. In the presence of either salt the monomers and tetramers occur in solution and their relative weight concentration depends on the number of ions per DNA molecule, not on DNA or salt concentration alone.

In the presence of either salt a small number of ions per DNA molecule favoures the formation of monomers, while a high one favoures the formation of tetramers. The main difference seems to be that the presence of potassium is more favourable for tetramer formation – the majority of DNA in the sample is transformed into the tetramer form at about 40 K^+ ions per DNA molecule and at about 100 Na^+ ions per DNA molecule. The results presented in Figs. 4 and 6 depend on the previous preparation of the sample, e.g. repeated cycles of thermal denaturation and cooling shift the equilibrium towards the tetramer.

In the presence of NaCl, Hardin *et al.* (1991) and Wang and Patel (1994) observed formation of monomers, while Chen (1992) reported formation of tetramers. The experimental data given by Wang and Patel (1994) and Hardin *et al.* (1991) imply that their measurements were conducted in solutions with the number of Na^+ ions per DNA molecule below 100, in which according to Fig. 6 the monomer should dominate. The Chen (1992) experiment by gel electrophoresis was performed at a concentration of Na^+ ions per DNA molecule above 100, at which the tetramer form should dominate. In view of the above findings, the results reported by those authors can be treated as consistent with the findings reported in this work. A similar comment can be applied to the results reported by Hardin *at al.* (1991) and Chen (1992) obtained by gel electrophoresis in the presence of KCl, indicating the dominant formation of tetramers. In our opinion this result is a consequence of the high number of K^+ ions per DNA molecule in both experiments. However, in the Chen study (1992) it could also be a consequence of thermal preincubation of the sample.

Results for Sr^{2+}

The measurements were performed for the equilibrium concentrations of $SrCl_2$ of 1 mM and 15 mM. In the presence of 1 mM $SrCl_2$ three diffusion processes were observed (Fig. 7a). One of them was characterised by the diffusion coefficient value extrapolated to zero concentration of DNA close to the value obtained for the monomer quadruplex, for another one, this value was close to that for the tetramer quadruplex, hence one of the diffusion modes was assigned to the monomers and the other to the tetramers. The diffusion coefficient characterising the third diffusion mode extrapolated to zero concentration of DNA was 0.2×10^{-6} cm^2/s, which corre-

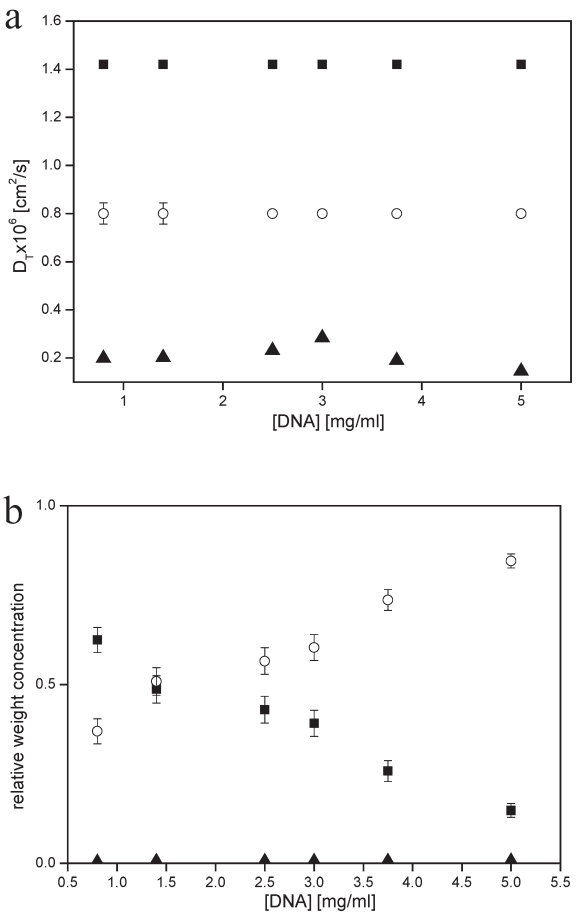

Figure 7. The measured translational diffusion coefficients and relative weight concentrations obtained in the presence of 1 mM $SrCl_2$.

a. The measured translational diffusion coefficient of the three forms of the telomere *versus* the telomere concentration at 1 mM $SrCl_2$. Symbols: monomer ■, tetramer ○, aggregate ▲. **b**. Relative weight concentrations of the monomer ■, tetramer ○, aggregate ▲ *versus* DNA concentration at 1 mM $SrCl_2$.

sponded to a structure whose size was estimated as σ = 100 Å and mass as 300 times that of the monomer. This structure was referred to as an aggregate. Figure 7b presents relative weight concentrations of the monomer, tetramer and aggregate *versus* DNA concentration. As follows from the Figure, the relative weight concentration of the aggregate is close to zero and the fact that its presence has been detected at all is due to a specific feature of the PCS method which is a scattering method so any signal from large structures dominates the correlation function even at a minimal concentration. Unfortunately, this also means that the results obtained for the monomer and tetramer are burdened with larger uncertainties. Figure 7b also implies that at high DNA concentrations the tetramer form dominates, while at low concentration — the monomer. For the DNA concentration of about 1.4 mg/ml the weight concentrations of both forms are the same. This behaviour is different than that observed in the presence of NaCl or KCl, in which a decreasing DNA concentration favoured the formation of tetramers. At a $SrCl_2$ concentration of 15 mM, three diffusion processes were observed. One was related to the tetramer quadruplex, and the other two to the dynamics of the structures whose translational diffusion coefficients extrapolated to zero DNA concentration were (Fig. 8a) 0.3×10^{-6} cm^2/s and 1.6×10^{-8} cm^2/s. The structures were referred to as aggregate 1 and aggregate 2. The hydrodynamic radius of aggregate 1 was estimated as σ = 70 Å, and its mass as about 67 times greater than that of the monomer. The hydrodynamic radius of aggregate 2 was estimated as σ = 10 μm, and its mass as 4.2×10^5 times greater than that of the monomer. Figure 8b presents relative weight concentrations of the tetramer, aggregate 1 and aggregate 2. The relative concentration of the tetramer is much higher at the $SrCl_2$ concentration of 15 mM than at 1 mM, also at 15 mM no monomer structures are detected. These results are in agreement with those reported by Chen (1992), who found that higher concentrations of Sr^{2+} ions favour the formation of tetramers at the expense of other structures. Thus, the formation of tetramers at the

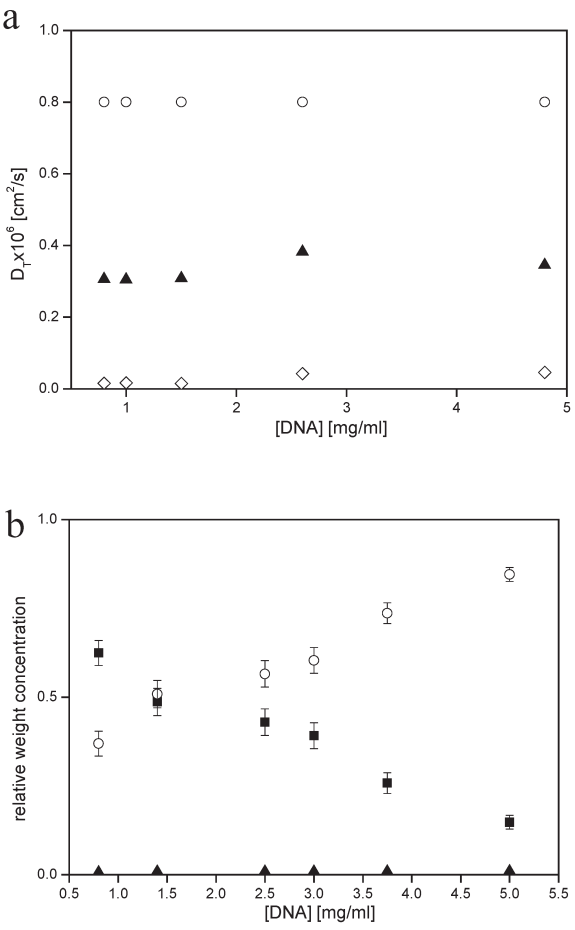

Figure 8. The measured translational diffusion coefficients and relative weight concentrations obtained in the presence of 15 mM $SrCl_2$.

a. The measured translational diffusion coefficient of the three forms of the telomere *versus* telomere concentration at 15 mM $SrCl_2$. Tetramer ○, aggregate 1 ▲, aggregate 2 ◇. **b**. Relative weight concentrations of the tetramer ○, aggregate 1 ▲, and aggregate 2 ◇ *versus* DNA concentration at 15 mM $SrCl_2$.

expense of monomers is favoured by increasing concentration of DNA (Fig. 7b) and by increasing concentration of Sr^{2+} ions. Therefore, it is pointless to describe the equilibrium in the presence of Sr^{2+} ions in terms of the number of ions per DNA molecule as was done for monovalent ions. As shown in

Fig. 8b, the relative weight concentrations of aggregate 2 is close to zero. High DNA concentrations favour the formation of aggregate 1 at the expense of tetramer, nevertheless, the tetramer dominates in the whole DNA range studied. On the basis of our results we can not predict what is the mechanism of the formation of aggregates in the presence of strontium, and whether this process is relevant to the G-wire self assembly as was previously reported by Marsh *et al.* (1995).

CONCLUSIONS

The telomeric sequence of *Tetrahymena thermophila* $d(TGGGGT)_4$ was studied by the PCS method in the presence of NaCl, KCl and $SrCl_2$. The sample was polydisperse under all conditions studied. The translational diffusion coefficients D_T describing the diffusion modes observed were determined. On the basis of a comparison of the experimental D_T values with those calculated theoretically using the bead model two of the forms were identified as the monomer and tetramer quadruplexes. The monomer and tetramer quadruplexes were observed in the presence of Na^+ and K^+ ions of all conditions studied and their relative weight concentrations were determined. These concentrations depended not only on the equilibrium salt concentration but also on the concentration of DNA. The results obtained in the presence of monovalent ions could be presented as a plot of the relative weight concentration of DNA *versus* the number of ions per DNA molecule. The presence of a high number of ions per DNA molecule favoured the formation of tetramer structures and a low number of ions per DNA molecule – the formation of monomer. A structural monomer-tetramer transition was induced by the change in the number of ions per DNA molecule. The effect of Na^+ ions on the formation of the quadruplex structures was similar to that of K^+, although the effect of K^+ ions was stronger. For NaCl the number of ions per DNA molecule at which the relative weight concentrations of the monomer and tetramer structures were the same was about 100, while for KCl it was about 40. In the presence of Sr^{2+} ions, besides the monomeric and tetrameric quadruplexes, formation of different size of aggregates was observed, with an average size of several micrometers. The size and mass of the aggregates were estimated and their relative weight concentrations were determined. Increasing Sr^{2+} concentration favoured formation of tetramers at the expense of monomers, and had a similar effect as the increasing concentration of DNA. The results obtained in the presence of Sr^{2+} ions could not be described simply in terms of the number of ions per DNA molecule. The effect of the presence of Sr^{2+} ions on the types of structures formed was also much stronger than that of the presence of monovalent ions.

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