Synchrotron radiation small angle scattering studies of d(TTAGGG)₄ oligomer in solution

Maciej Kozak, Agnieszka Włodarczyk, Andrzej Dobek

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1. Introduction

The eukaryotic chromosomes are terminated with the specialized DNA structures, known as telomeres. They are made of small, tandemly repeated, guanidine-rich DNA sequences. Telomeres vary little in sequence (e.g., TTGGGG in Tetrahymena, TTAGGG in human) but significantly in length (ranging from about 50 bp in Euplotes to over 100 kbp in mice) (König and Rhodes, 1997). They form specific four-stranded structures called G-quadruplexes with a core made up of guanines stabilized by Hoogsteen type cyclic hydrogen bonds. Telomeric sequences play a crucial role in maintaining the eukaryotic chromosome specifically bound to the structural proteins within the cell, which prevents the irregular recombination, the nucleolytic degradation, the fusion or other events lethal to the cell. The ends of chromosomes are progressively cut down on each replication cycle. This process seems to be linked with the limited proliferative ability of normal somatic cells. Probably the loss of the telomeric tandem leads to the cell death (Rhodes and Giraldo, 1995; Wellinger et al., 1996). Therefore, the G-quadruplexes formed in human telomeres are promising anticancer targets (Neidle and Parkinson, 2002).

The structure and the conformation of G-DNA have been investigated by X-ray crystallography (Haider et al., 2002; Parkinson et al., 2002) and NMR spectroscopic techniques (Phan et al., 2007; Wang and Patel, 1993; Matsugami et al., 2007). The G-DNA quadruplexes can adopt different folding patterns and stoichiometries: tetrameric structure with all strands parallel, dimeric antiparallel structure with adjacent parallel strands, monomeric antiparallel structure with alternating parallel strands, monomeric antiparallel structure with three double chain reversal loops, monomeric antiparallel structure with adjacent parallel strands and a diagonal loop, monomeric mixed structure with three parallel and one antiparallel strands. The last three monomeric structures have been observed in the human telomeres (Huppert, 2008).

The aim of our studies was the characterisation of low-resolution structure and potential conformational changes of a synthetic d(TTAGGG)₄ oligomer in solution in the absence and in the presence of potassium cations.

2. Materials and methods

The small angle X-ray scattering measurements were performed on the X33 camera (Boulin et al., 1988) of EMBL on DORIS storage ring at DESY, Hamburg. The measurements were made using a linear gas proportional detector with delay line readout. The measurements were carried out on a series of d(TTAGGG)₄ oligomer solutions in 10 mM Tris/HCl pH 7.3 (concentrations 2, 4, 6, 8 and 10 mg/ml) in 10 mM Tris/HCl pH 7.3, 0.1 mM KCl (concentrations 3, 5, 7 and 10 mg/ml) in 10 successive 60 s frames. The samples were placed in a 1 mm cell (100 µl volume) with mica windows (20 µm) and at 10 °C. The sample-to-detector distance was 1.7 m, covering the s-axis range 0.12 > s > 4.4 nm⁻¹ (where s = 4π sinθ/λ, with θ the scattering angle and the X-ray wavelength λ = 0.15 nm). The s-axis was calibrated using the...
diffraction patterns of turkey tendon collagen and tripalmitin (Bear, 1944; Kellens et al., 1991). The SAXS data were corrected for detector response, normalized to the incident beam intensity, the scattering of the buffer was subtracted and the final scattering curve was obtained by merging the scattering data collected at a lower concentration and higher concentration using the program package PRIMUS (Konarev et al., 2003).

The radii of gyration \( R_G \) characterising the molecules in solutions were evaluated using the Guinier equation (Guinier, 1939) and from the full scattering curve using the indirect transform program GNOM (Svergun, 1992). The theoretical scattering curves from the X-ray structure and the homology models were calculated using the program CRYSOL (Svergun et al., 1995).

On the basis of the SAXS data, the low-resolution structure in solution was reconstructed using \( ab \) initio methods and the program DAMMIN v5.3 (Svergun, 1999).

### 3. Results and discussion

The SAXS data collected for different \( d(TTAGGG)_4 \) oligomer solutions (in the absence and in the presence of potassium cations) are presented in Fig. 1a. The radius of gyration \( R_G \), calculated for \( d(TTAGGG)_4 \) oligomer (in 10 mM Tris/HCl pH 7.3) was 1.42 nm, while that for \( d(TTAGGG)_4 \) oligomer (in 10 mM Tris/HCl pH 7.3; 0.1 mM KCl) was 1.32 nm. The intramolecular pair distance distribution functions and the maximum size of the particles in solution \( D_{\text{max}} \) were estimated using the program GNOM and are presented in Fig. 1b. The pair distance distribution functions, \( P(r) \), yielded a maximum size of the particles in the solutions in the absence and in the presence of potassium cations as \( D_{\text{max}} = 4.55 \) and 4.35 nm, respectively.

Several crystal and NMR structures of G-DNA oligomers with sequences similar to \( d(TTAGGG)_4 \) were compared with experimental data. The best fit presented in Fig. 1a was obtained for the NMR structure of the parallel-stranded DNA quadruplex TAGGG(TTAGGG)_3 (PDB code: 2JSM; Phan et al., 2007). The comparison reveals noticeable systematic deviations. The discrepancy between the theoretical model (PDB: 2JSM) and the experimental curve for \( d(TTAGGG)_4 \) solutions in the absence of...
potassium cations and the data calculated from the NMR structure of \( \chi = 2.129 \) was obtained for the excluded volume \( V = 5.32 \text{nm}^3 \) and \( \delta \rho = 133 \text{e/nm}^3 \) (where \( \delta \rho \) is the contrast in the solvation shell) corresponding to the hydration level 0.777 (w/w). However, for d(TTAGGG)₄ solutions in the presence of potassium cations the best agreement between the experimental and the calculated scattering curves (the discrepancy between the data \( \chi = 1.301 \)) was obtained for the excluded volume \( V = 5.43 \text{nm}^3 \) and \( \delta \rho = 107 \text{e/nm}^3 \) corresponding to the hydration level 0.733 (w/w). The above differences can be attributed not to the differences in the sequence of the DNA model used for comparison but rather to the influence of different hydration degrees and also to the solvation of K⁺ cations stabilizing the structure.

To obtain independent information about the low-resolution structure of the molecule in solution, the shapes of d(TTAGGG)₄ oligomer were restored by the ab initio calculations from both experimental data sets.

The ab initio shape reconstruction was made inside the search volume of the diameter \( D_{\text{max}} = 4.35–4.55 \text{nm} \) filled with 1400–1900 dummy atoms. The packing radius of the dummy atoms was 0.14 nm and the total number of non-solvent dummy atoms was 500–700. The reconstruction of the molecular shape was made without symmetry restrictions and assuming a prolate or unknown geometry of the molecule that would allow a neat fit of the experimental data. The typical solutions obtained from DAMMIN are displayed in Fig. 2.

The radii of gyration and \( D_{\text{max}} \) values characterising the d(TTAGGG)₄ oligomer models in solution at pH 7.3 were: \( R_g = 1.42 \text{nm} \) and \( D_{\text{max}} = 4.53 \text{nm} \) in the absence of potassium cations and \( R_g = 1.32 \text{nm} \) and \( D_{\text{max}} = 4.28 \text{nm} \) in the presence of potassium cations. The structure of d(TTAGGGTTAGGG)₃ selected for the comparison is a unique form of the human telomere containing the (3+1) G-quadruplex core (Phan et al., 2007). The DAMMIN models relatively well reproduce the shape of the DNA quadruplex. The models obtained on the basis of the SAXS data in the presence of potassium cations are more compact and similar to the reference model (see Fig. 2c). However, the models of d(TTAGGG)₄ oligomer in solution in the absence of K⁺ cations are elongated and less compact.

4. Conclusions

The comparison of SAXS data with the NMR structure of the parallel-stranded DNA quadruplex TAGGG(TTAGGG)₃ (PDB code: 2JSM) reveals noticeable systematic deviations. The discrepancy between the theoretical model (PDB: 2JSM) and the experimental SAXS curve for d(TTAGGG)₄ solution in the presence of potassium cations is lower than for scattering data collected in the absence of potassium cations.

The low-resolution models of d(TTAGGG)₄ solution obtained on the basis of the SAXS data relatively well reproduce the shape of the DNA quadruplex. The DNA oligomer structure in solution in the presence of potassium cations is more compact and similar to the reference NMR structure.

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