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Structural analysis of thrombin-binding G-aptamers in presence of bivalent ions

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Abstract. The aim of this study was to examine 3D structures of DNA aptamers, thrombin inhibitors. •The main objective was to study 3D structure 15TBA, RE31, NU172 aptamers using the small-angle X-ray scattering method. The size of 15TBA was 4.5 nm, which corresponds to a partially unfolded conformation. The CD spectrum of Nu172 in the presence of 50 mM strontium ions indicates the presence of an antiparallel G-quadruplex, the concentration o f which drops at 50°C. NU172 does not have a rigid structure, apparently due to the presence of a guanine residue in the GT loop. The NU172 aptamer does not form a stable conformation in solution either without ions or with Ba2+ and Sr2+ ions. • It was shown that there is possibility of aptamers transition from one conformation to another dependently on concentration and temperature confirms that the potassium ion is a unique stabilizing ion of natural molecules containing G-quadruplexes.

Key words: 3D structures, DNA aptamers, thrombin inhibitors, G-quadruplexes.
Conflict of interest. The authors declare the absence of obvious and potential conflicts of interest associated with the publication of this article.
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One of the popular areas in molecular medicine is a construction and application synthetic oligonucleotides (including modified) – aptamers. Aptamers possess ability of forming non-standard DNA structures, which play vital role in regulations of cell processes. One of such conformations are G-quadruplexes consisting of several G-tetrads (fig.1).

Small-angle X-ray scattering (SAXS) method allows to obtain extensive information about biomolecules, such as information about size and shape of macromolecules, intermolecular association, domains and linker flexibility, ligands binding. SAXS data is used in combination with alternative methods for studying structure of macromolecules (e.g. X-ray crystallography, circular dichroism (CD), molecular dynamics and metadynamics), but it also can be used as an independent method.

In this study, thrombin-binding aptamers 15TBA [1], RE31 [2], NU172 [3], were examined (table). These aptamers structurally belong to a same class of aptamers, G-quadruplex aptamers. Therefore, some structural similarities can be identified, in particular, presence of following domains: duplex, transition and G-quadruplex domains as well as presence of dinucleotide loops (TT or TG) and trinucleotide loop.

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The aim of this study was to examine 3D structures of DNA aptamers, thrombin inhibitors. The main objective was to study 3D structure 15TBA [1], RE31 [2], NU172 [3] aptamers using the SAXS method (fig.1). Structure of these aptamers has been discovered earlier using X-Ray crystallography method [1, 3, 4], however, structure in solution and in crystal might differ. Experiments were carried out at the BioMUR beamline in the National Research Center "Kurchatov Institute" at the temperatures 5°C, 20°C, 38°C, 45°C, and 50°C. Concentrations of barium and strontium were varied from 0mM to 50mM (0mM,

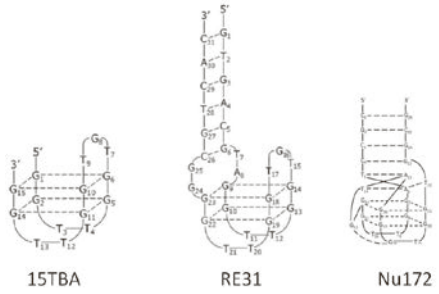

Figure 1. Structure of DNA aptamers to thrombin.

0.5mM, 5mM, 50mM) for all aptamers (fig.2). A) Pair distance distribution function (PDDF) p(r) based on the SAXS data of 15TBA without ions at various temperatures from 5°C to 45°C. At 5-38°C the structure is more compact. B) p(r) function based on the SAXS data of 15TBA in buffer with 50 mM Barium ion at various temperatures. It is observed the average compact structure of 15TBA at all temperatures with the slight decreasing the folded state. C) p(r) based on the SAXS data of 15TBA in buffer with 50 mM Strontium ion at various temperatures. SAXS data about the aptamer structure shows the similar signal, the maximum size D_{max} fluctuates in the range 4.5-5.5 nm. D) CD of 15TBA. The lines show the results of the CD of 15TBA in a buffer without ion at different temperatures from 5°C up to 50°C. There is signal which corresponds to antiparallel G-quadruplex. Signal almost fully disappears at 20°C. E) CD of 15TBA. The green lines show the results of the CD of 15TBA in a buffer with 50 mM strontium ion at different temperatures from 5°C up to 50°C. There is signal which corresponds to both parallel and antiparallel G-quadruplex. The black line shows the result of the CD of 15TBA in a buffer with 50 mM Barium ion at different temperatures from 5°C. F) CD of 15TBA. The lines show the re-

Table

Sequence of DNA aptamers to thrombin

Aptamer	Sequence
15TBA	GGTTGGTGTGGTTGG
RE31	GTGACGTAGGTTGGTGTGGTTGGGGCGTCAC
NU172	CGCCTAGGTTGGGTAGGGTGGTGGCG

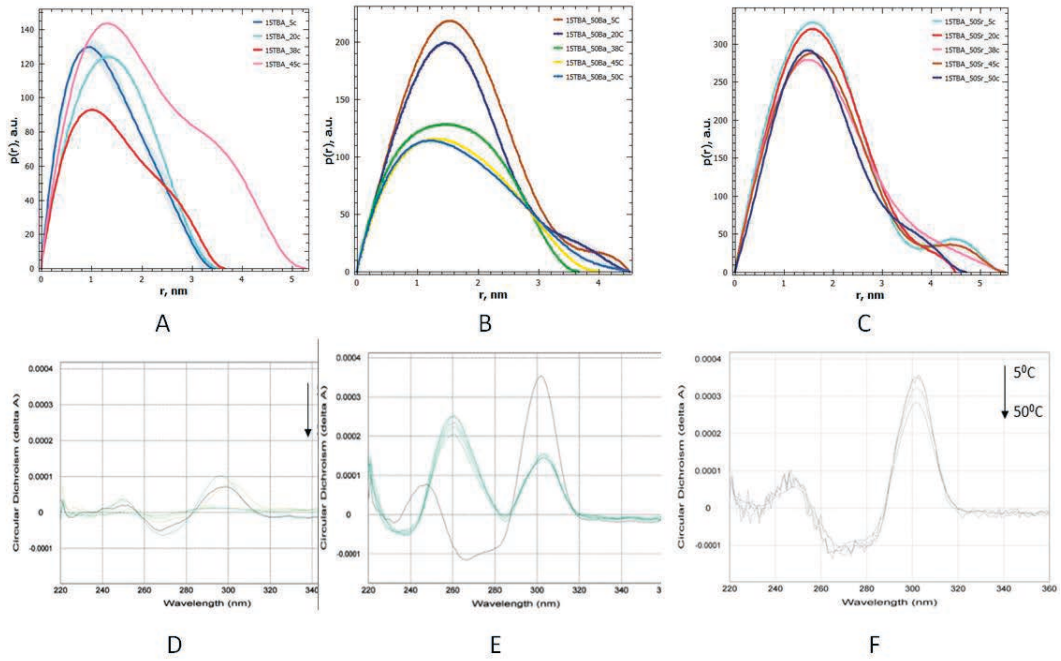

Figure 2. SACS and circular dichroism data DNA aptamers to thrombin (description in text).

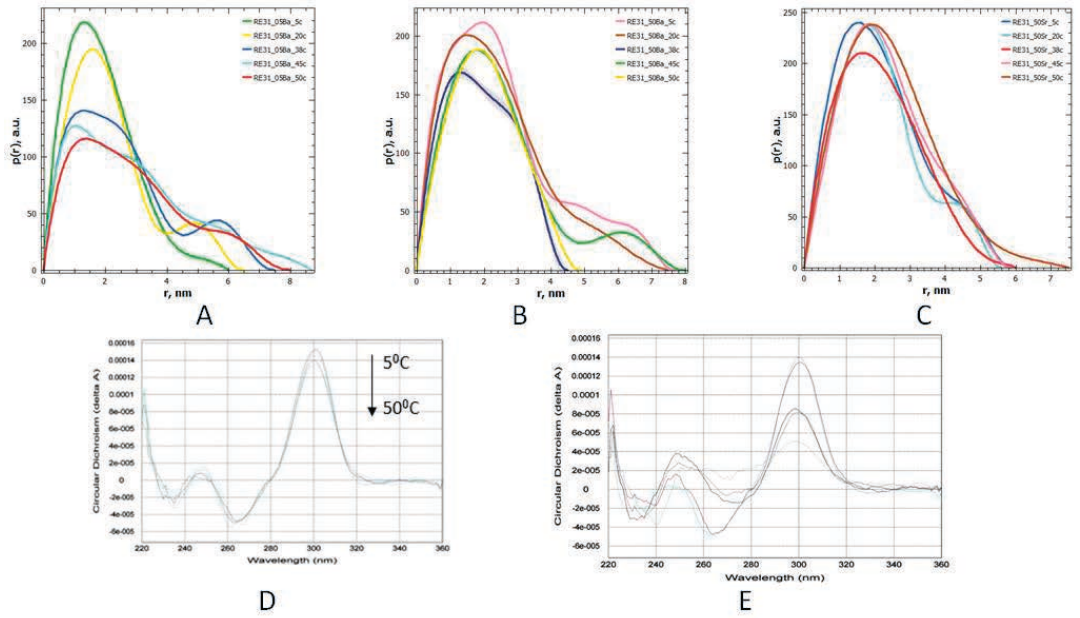

Figure 3. SACS and circular dichroism data DNA aptamers RE31 (description in text).

sults of the CD of 15TBA in a buffer with 50 mM Barium ion at different temperatures from 5°C up to 50°C.

According to the SAXS data, the size of 15TBA was 3.5-4.5 nm, and melting the aptamer is more distinguish without ions in the buffer (fig.3). A) PDDF based on the SAXS data of RE31 without ions at various temperatures. Melting point is observed at the temperatures 38°C and higher. At 5-20°C structure is more compact. B) PDDF based on the SAXS data of RE31 in buffer with 50 mM Barium ion at various temperatures. There is a probability of oligomerization of the aptamer, since the different D_{max} values are observed at the different temperatures. C) PDDF based on the SAXS data of RE31 in buffer with 50 mM Strontium ion at various temperatures. While the RE31 aptamer melts at about 38°C without ions, its melting point is shifted in presence of Strontium ions to 50°C. D) CD of RE31. The lines show the results of the CD of RE31 in a buffer with 50 mM Barium ion at different temperatures from 5°C up to 50°C. There is signal which corresponds to antiparallel G-quadruplex. E) CD of RE31. The lines show the results of the CD of RE31 in a buffer with 50 mM Strontium ion at different temperatures from 5°C up to 50°C. There is signal which corresponds to antiparallel G-quadruplex.

CD spectrum of RE31 in the presence of 50 mM strontium ions indicates presence of an antiparallel G-quadruplex, concentration of which drops at 50°C.

According to the results of the SAXS experiment, aptamer showed has two different sizes, 6 and 7.5-8 nm, which approximately corresponds to the G-quadruplex conformation for 6 nm (the slight discrepancy in sizes between SAXS and X-ray Diffraction (XRD) data is most likely due to the fact that the movement and flexibility of molecule in solution is not restricted, while in the crystal it is rigidly fixed). The second size variation - 7-8 nm - corresponds to a partially unfolded conformation (fig.4). A) PDDF p(r) based on

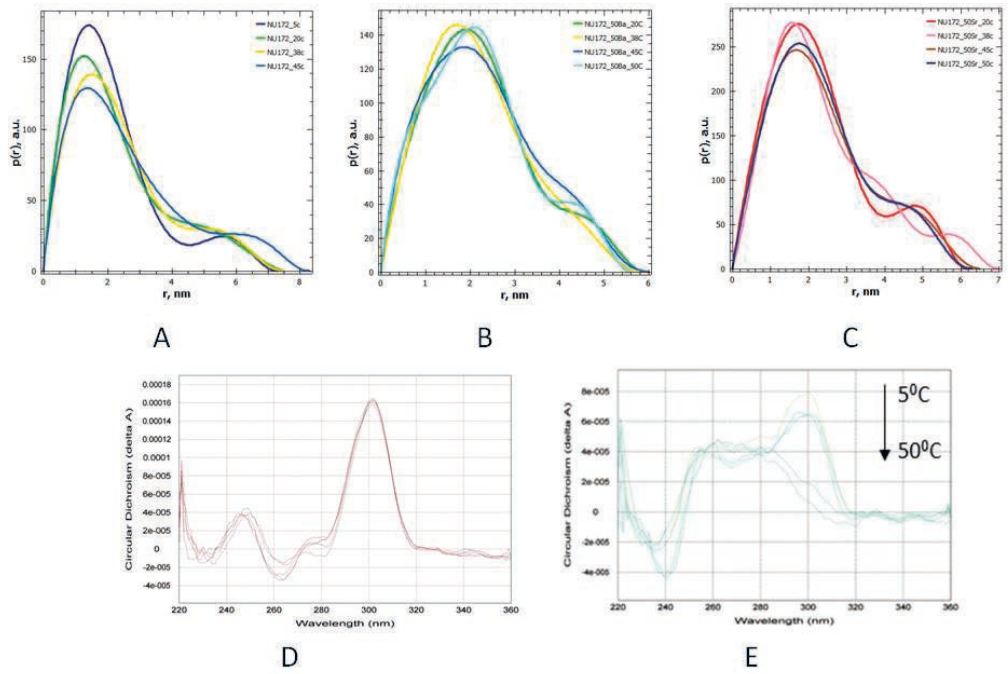

Figure 4. SACS and circular dichroism data DNA aptamers Nu172 (description in text).

the SAXS data of Nu172 without ions at various temperatures. All the results at 5°C - 45°C show unfolded or partially unfolded state of the molecule without ions in the buffer. B) PDDF based on the SAXS data of Nu172 in buffer with 50 mM Barium ion at various temperatures. The partially unfolded conformation of Nu172 is preserved at all temperatures with slight increasing the flexibility at higher degrees. D) PDDF based on the SAXS data of Nu172 in buffer with 50 mM Strontium ion at various temperatures. All curves correspond to the partially unfolded conformation of the aptamer molecule with higher value D_{max} in comparison to the SAXS curves for RE31 with Ba ions in the buffer. E) CD of Nu172. The lines show the results of the CD of Nu172 in a buffer with 50 mM Barium ion at different temperatures from 5°C up to 50°C. There is signal which corresponds to antiparallel G-quadruplex. E) CD of Nu172. The lines show the results of the CD of Nu172 in a buffer with 50 mM Strontium ion at different temperatures from 5°C up to 50°C. There is signal which corresponds to antiparallel G-quadruplex; however, it significantly weakens at 20°C.

The CD spectrum of Nu172 in the presence of 50 mM strontium ions indicates the presence of an antiparallel G-quadruplex, the concentration of which drops at 50°C.

NU172 does not have a rigid structure, apparently due to the presence of a guanine residue in the GT loop, the average size according to SAXS data was 6-7 nm, which corresponds to a partially unfolded conformation. According to the SAXS results, the NU172 aptamer does not form a stable conformation in solution either without ions or with Ba^{2+} and Sr^{2+} ions.

Conclusions

1. Using SAXS method, the sizes of the molecules included in these complexes were characterized.

2. Circular dichroism spectra shows presence of antiparallel quadruplexes at a certain concentration of barium and strontium ions at low temperatures.

3. Aptamers thermal stability has been examined and existence of a set of conformers, which are both quadruplex structures and partially unfolded molecules, has been showed.

4. It was shown that there is a possibility of aptamers transition from one conformation to another dependently on concentration and temperature confirms that the potassium ion is a unique stabilizing ion of natural molecules containing G-quadruplexes.

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