UDC 577.22

DOI: 10.20333/2500136-2021-2-76-78

Structure approaches to study of DNA aptamers in solution

R. V. Moryachkov[1,2], P. A. Nikolaeva[3], V. A. Spiridonova[4]

[1] Federal Research Center "Krasnoyarsk Science Center SB RAS", Krasnoyarsk 660036, Russian Federation

[2] Kirensky Institute of Physics, Krasnoyarsk 660036, Russian Federation

[3] Lomonosov Moscow State University, Moscow 119992, Russian Federation

[4] A. N. Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow 119992, Russian Federation

Abstract. The high potential of aptamers – specific molecular agents based on short single-stranded nucleic acids – makes high demands on the molecules under development for the efficiency of interaction with target biomolecules. In this work, approaches are considered for studying the spatial structure of DNA aptamers in solution using various complementary methods, which make it possible to obtain a more complete picture of the formation of the structure and conformational changes, to track the interaction with the target protein, the tendency to oligomerization, and to characterize the spatial structure of both individual molecules and complexes.

Key words: biomolecules in solution, tertiary structure, small-angle X-ray scattering (SAXS), structure analysis.

Conflict of interest. The authors declare the absence of obvious and potential conflicts of interest associated with the publication of this article.

Citation: Moryachkov RV, Nikolaeva PA, Spiridonova VA. Structure approaches to study of DNA aptamers in solution. *Siberian Medical Review.* 2021; (2): 76-78. DOI: 10.20333/2500136-2021-2-76-78

Introduction

Research and development of various DNA and RNA aptamers, as well as the active development of methods for their selection, modification, and application have shown the effectiveness of such molecular agents for targeted drug delivery, imaging and therapy of various diseases [1,2]. For a more careful selection by the binding site of the recognizing agent, it is necessary to determine the most probable conformation of the aptamer. To address this issue, X-ray diffraction analysis is the standard method for restoring the three-dimensional structure of biomolecules. However, only a few developed aptamers were crystallized in the complex with their target proteins; for most of such objects this is a difficult task. The most effective approach is to extract useful information about the structural features of a biomolecule from various experimental data. This paper presents approaches to the utilization of small-angle X-ray scattering (SAXS) method, size-exclusion chromatography (SEC), and circular dichroism (CD), whose data are used to reconstruct the spatial structure of the aptamer in solution.

Material and methods

For the study, we used a 110 DNA aptamer to interleukin-6 with a sequence length of 31 nucleotides and a molecular weight of 9.78 kDa. The aptamer was prepared in a Tris-HCl buffer solution with a concentration of 9.8 mg / ml in a volume of 80 μl, that is sufficient for its dilution in a GE Superdex 75 Increase 10/300 chromatography FPLC column in a volume of 24 ml and registration of scattered X-ray radiation. The SAXS measurements on the aptamer were carried out at the P12 BioSAXS beamline of the Petra III synchrotron (DESY, Hamburg, Germany). The X-ray radiation wavelength at the station is 0.124 nm, which corresponds to an energy of 10 keV, the sample-detector distance is 3.0 meters, and the exposure duration for each frame is 1 sec.

Initially the buffer solution in a volume of 48 ml was passed through the chromatographic column in the Agilent 1260 Infinity instrument at a pressure of 21.1 bar. Then, a solution with the sample was added to the column under the same pressure at a temperature of 20.4 °C. After passing through the column, the solution automatically enters the setup for SAXS measurements using synchrotron radiation supplied from the beamline of the synchrotron storage ring. In parallel with the dilution of the solution using liquid size exclusion chromatography, the installation measures the level of optical absorption of ultraviolet radiation to determine the concentration of oligonucleotides in the solution. Measured data were processed using the standard SAXS pipeline [3] in the program suite ATSAS [4].

Results and discussion

Joint SEC-SAXS measurements were carried out together with UV absorption on a DNA aptamer 110 to interleukin-6 in solution (figure 1) [5]. As a result we obtained data on the concentration of the aptamer in solution during the flowing through the chromatographic column. We found out that the solution contains at least two distinguished fractions with the sample, which differ in the mass of particles. The third minor intermediate fraction was rather weakly expressed for a good-quality SAXS signal to be obtained.

Using the SAXS method we characterized particles from both main fractions separately. As a result, the following structural data were obtained: the distribution of the molecular mass relative to the center of mass – radius of gyration, maximum particle size and total volume of the electron density of the whole molecule, distance distribution functions were constructed. With the knowledge

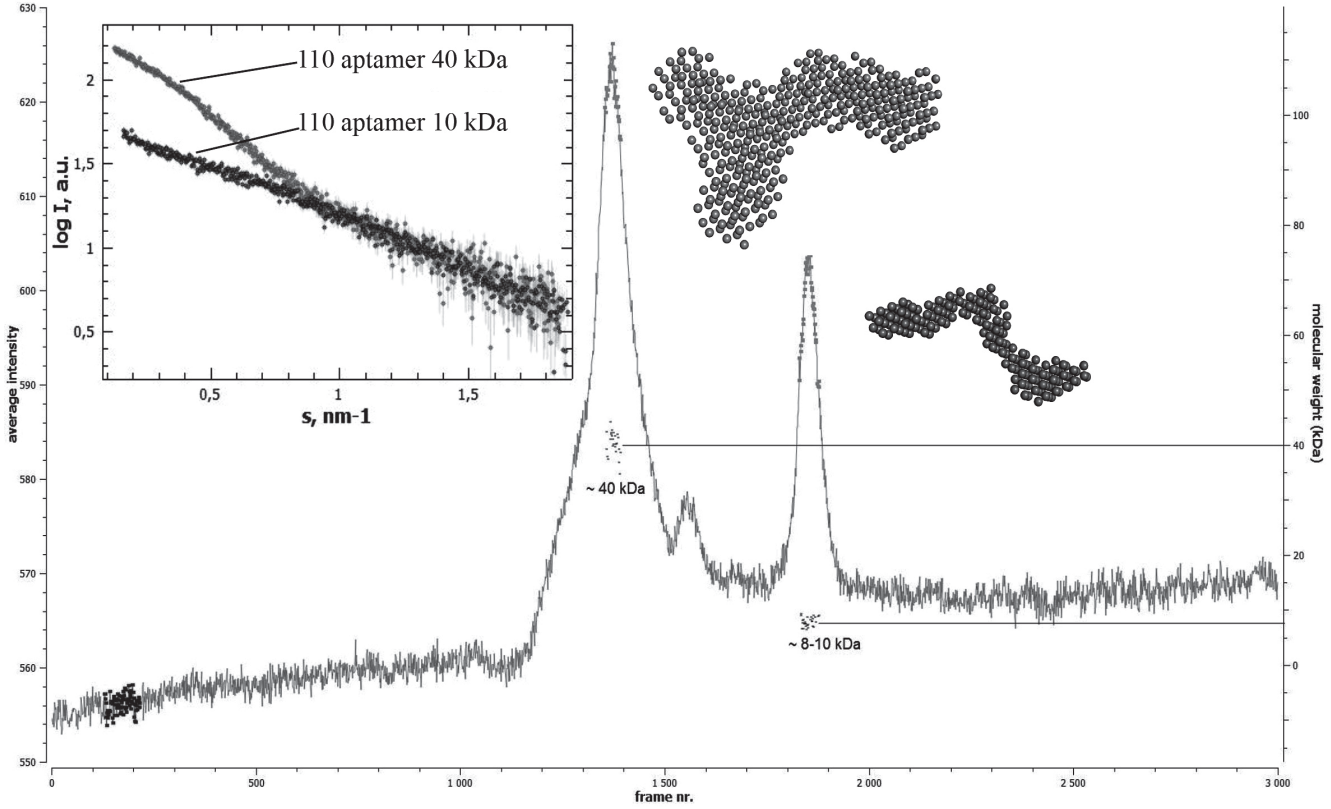

Figure. Main graph – the dependence of the intensity of UV absorption of the aptamer depending on the time of flowing the solution through the chromatographic column. Bead models above the graph are spatial forms of the tetramer and monomer of the aptamer molecule corresponding to particles with masses of 40 and 10 kDa, respectively. Inserted graph – SAXS plots for each of the solution fractions processed separately.

of the average density of the DNA molecule it is possible to calculate the approximate value of the mass of the molecule: in the first fraction, the particles have a mass of about 40 kDa, which corresponds to four times the mass of the molecule, in the second – 8-10 kDa, which corresponds to the mass of the monomer. Analysis of the SAXS data also allowed us to determine that aggregation of molecules to larger constituents is not observed in both fractions, since this would introduce noticeable changes in the scattering patterns, which can be determined using analytical methods such as Guinier analysis. For the range $s > 1$ nm^{-1}, SAXS plots have an almost similar shape, which is associated with the repetition of the structure on the scale of nucleotides and domains of the molecule, and the difference at small angles indicates a difference in the structure on the scale of the entire molecule and its tertiary structure.

Reconstruction of the spatial shape of low-resolution molecules, as well as analysis of the distance distribution functions for data from each fraction, showed that the monomer molecule has an elongated structure, which is most likely associated with the presence of a free single-stranded DNA chain and elements of a double helix in the structure. The larger structure of the tetrameric component in solution may indicate a more complex structure, for example, the presence of quadruplexes. Their presence is just confirmed by the data from CD spectroscopy, which reveal similar signals from the structural elements of DNA molecules.

Conclusion

Extracting data on biomolecules from several experimental methods allows one to look at them from different angles and fill the missing gaps in information about the behavior and structure of an object, help in developing a model of the object under study, and improve its predictive properties. Using the example of studying the DNA aptamer 110, we showed that various kinds of information on the behavior and shape of a molecule in solution makes it possible to obtain a more complete picture of the studied molecule and use complementary data to construct the full-atomic structure of a molecule, which can be used for the methods of molecular dynamics and docking with target protein.

The reported study was funded by RFBR, project number 19-32-90266.

References

1. Wu X, Chen J, Wu M, Zhao JX. Aptamers: Active Targeting Ligands for Cancer Diagnosis and Therapy. *Theranostics.* 2015;5(4):322–44. DOI: 10.7150/thno.10257

2. Zamay T, Zamay G, Kolovskaya O, Zukov R, Petrova M, Gargaun A. Current and Prospective Protein Biomarkers of Lung Cancer. *Cancers*. 2017;9(12):155. DOI: 10.3390/cancers9110155

3. Da Vela S, Svergun DI. Methods, development and applications of small-angle X-ray scattering to characterize biological macromolecules in solution. *Current Research in Structural Biology*. 2020;(2):164-70. DOI: 10.1016/j.crstbi.2020.08.004

4. Manalastas-Cantos K, Konarev PV, Hajizadeh NR, Kikhney AG, Petoukhov MV, Molodenskiy DS, et al. ATSAS 3.0 : expanded functionality and new tools for small-angle scattering data analysis. *Journal of Applied Crystallography*. 2021;54(1):343–55. DOI: 10.1107/S1600576720013412

5. Graewert MA, Da Vela S, Gräwert TW, Molodenskiy DS, Blanchet CE, Svergun DI. Adding Size Exclusion Chromatography (SEC) and Light Scattering (LS) Devices to Obtain High-Quality Small Angle X-Ray Scattering (SAXS) Data. *Crystals*. 2020;10(11):975. DOI: 10.3390/cryst10110975

Author information

Roman V. Moryachkov, researcher, Federal Research Center «Krasnoyarsk Science Center SB RAS»; Address: 50, Akademgorodok St., Krasnoyarsk, Russian Federation 660036; Phone: +7 (391) 243-45-12; researcher, Kirensky Institute of Physics; Address: Bld. 38, 50, Akademgorodok St., Krasnoyarsk, Russian Federation 660036; Phone: +7(391)2432635; e-mail: mrv@iph.krasn.ru, http://orcid.org/0000-0002-0409-779X

Polina A. Nikolaeva, researcher, Lomonosov Moscow State University; Address: 1, Leninskie Gory St., Moscow, Russian Federation 119992; Phone: +7(495)9391000; e-mail: nikolaevapa@gmail.com

Vera A. Spiridonova, Dr. of Biol. Sc., A.N. Belozersky Institute Of Physico-Chemical Biology, Lomonosov Moscow State University, Bld. 40, 1, Leninskie Gory St., Moscow, Russian Federation 119992; Phone: +7(495)9391000; e-mail: spiridon@belozersky.msu.ru

Received 17 February 2021
Revision Received 17 March 2021
Accepted 31 March 2021