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Small-Angle Scattering Applications to the Analysis of Aptamer Structure and Conformational Changes

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Abstract. Aptamers, structured single-chain oligonucleotides, are promising tools for detection of a wide variety of compounds, from high to low molecular weight, and affecting on them. The aptamers that are most affine for a detectable compound are selected from the libraries of random sequences by the SELEX method (Systematic evolution of ligands by exponential enrichment). The reason why aptamers deserve a special consideration lies in the specific features of their structure and the mechanism of binding to their target. Aptamers can be exploited for metal-ion sensing, biosensing, drug delivery and other functions. To apply the oligonucleotides in the medicine, ecology, food production, agriculture, etc., we need to know how the aptamers bind to their targets, how they change their conformation upon specific binding and how the environment influences on the affinity of aptamers. Small-Angle X-ray scattering showed that the interaction of aptamers with heavy metal and other divalent ions proceeds according to different mechanisms, and the aptamers used undergo different conformational changes.

INTRODUCTION

Interest in aptamers as specific receptor molecules is also due to the presence of various functional groups that can bind to heavy and transition metal ions [1]. This property contributed to the development of the field of analytical chemistry, in which interactions and the possibility of using aptamers in biosensors for the purpose of environmental monitoring are studied [2]. Both linear aptamers and those forming certain structures (quadruplex, hairpin, loop) are used to develop analytical test systems. Wang et al. [3] applied the aptamer 5′-SH-(CH2)10-CTTCTTTCTTCCCCC-TTGTTTGTTG-FAM-3′ that forms a "hairpin" under the interaction with mercury ions. The method of detection is based on energy transfer from the fluorescent label at the end of the aptamer to the surface of the gold nanoparticle as a carrier. Li et al. [4] used the conjugate of gold nanoparticles and aptamer forming the G-quadruplex structure for detection of lead ions.

Various detection methods (colorimetry, fluorescence, electrochemistry) in combination with nanoscale carriers make it possible to detect a change in the analytical signal during the interaction of the aptamer-nanoparticle conjugate with heavy metal ions [5-7]. However, despite the widespread use of such oligonucleotide sequences, there is a certain lack of information on the mechanisms of interaction and selectivity, how aptamers are able to change their structure when interacting with metals, and how the concentration of such a low molecular weight analyte affects the final conformation parameters.

Aptamers to cancer cells are used for diagnostics and therapeutics of the cancer [8-10], elicit the presence of cancer cells in the tissues and human blood [11-13], exhibit the catalytic activity by inhibition the function of target proteins [14,15], and also be the agents for drug delivery [16-18]. To use these functions, it is important to know how the aptamers bind with their targets, whether they change the conformation upon this binding, how the delivered "cargo" influences aptamer structure and therefore affinity and specificity. It excites also a great interest, whether we can and

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how to make shorter our aptamer avoiding the loss of its function. It needs a knowledge about the active site of the aptamer.

Aptamers are frequently long (respectively their width) and flexible molecules, that is one of the reasons why it is almost impossible to crystallize and study them alone (not in the complex aptamer-protein) by X-ray diffraction method. Besides this the structure of aptamer in solution influenced by the ions in the environment of the molecule attracts more interest in comparison to the crystal state.

In this work the Small-Angle X-ray Scattering (SAXS) method was applied to study the spatial structure of DNA aptamers [19]. This method allows to study the spatial molecule structure on the scale from 0.5 nm to hundreds of nm, in native environment, to track the conformational changes under varying conditions (temperature, pH, ion composition of the solution, during binding process with the molecular targets of aptamers and so on) directly in solution [20,21]. SAXS is actively used for protein structure investigation and became interesting from recently for studying DNA/RNA oligonucleotide and aptamer-protein complex structures [22-24].

We show some results of SAXS observations of the conformational changes for three DNA aptamers in solution, one of which is selected to the brain tumor cells and two to the heavy metal ions of lead and mercury.

MATERIALS AND METHODS

Standard water solutions of lead (II) and mercury (II) ions (1 g/L) stabilized with 0.1 M HNO3 were from Center of Standardization of Samples and High-Purity Substances (St. Petersburg, Russia). The (SH-C6)-CC-CCC-CCC-CCC-CCC (PolyC) and (SH-C6)-GGGTG GGTGG GTG (GT) oligonucleotides, aptamers, were synthesized by Syntol (Moscow, Russia). To prepare aqueous solutions of ions and aptamers deionized water, with a resistance of 18.3 MΩ • cm at 22 °C, was obtained using a Milli-Q Simplicity system produced by Millipore (Bedford, MA, USA).

Table 1. DNA aptamers synthesized for SAXS measurements.

Name of aptamer	Sequence	Number of nucleotides	MW	Target
Gli-233	ACTAT TCCAC TGCAA CAACT GAACG GACTG GAA	33	10.1	Glioblastoma cancer cells
GT	(SH-C6)-GGGTG GGTGG GTG	13	4.14	Pb^{2+}
PolyC	(SH-C6)-CCCCC CCCCC CCCC	14	3.99	Hg^{2+}

A characteristic feature of aptamers for lead ions is guanine-enriched sequences [25], and for mercury ions – thymine-enriched sequences [26-28]. This is why guanine-enriched sequences are more often used as sensing molecule for lead ions [29, 30]. In the same time high affinity of cytosine hydrogen bonds and similarity of functional groups to guanine [31,32] cause interest to cytosine as an aptamer-formed oligomer for lead ions detection. The use of guanine and thymine enriched aptamers for the detection of lead and mercury ions in water samples has been previously demonstrated [33]. The cross-linking interaction led to the aggregation of nanoparticles modified with this aptamer and a change in the colloid color.

DNA aptamer Gli-233 was selected to the glioblastoma cancer cells by the tissue-SELEX method [34]. Lyophilized aptamer was provided by IDT (Integrated DNA Technologies, USA). Samples were prepared in two solutions: PBS and PBS with Ca^{2+} and Mg^{2+} at 3 concentrations for each buffer: 0.8, 4.0 and 8.2 mg/mL.

SAXS measurements for the Gli-233 were performed on the beamline BM29 BioSAXS, ESRF in the Batch Mode, sample-to-detector distance was 2.85 m, X-ray wavelength - 0.099 nm, the store and measurement temperature for samples and buffer was 4°C.

The measurements of scattering the X-rays on the DNA aptamers GT and PolyC in water solution were carried out on the BioSAXS beamline at the Kurchatov Institute, Moscow. The wavelength of the X-rays was 0.145 nm, sample-to-detector distance - 30 cm.

We used solutions at room temperature. The aptamer was dissolved in 30 uL of water from the freeze-dried state to a concentration of 3 mg/mL. The first measurement was carried out in deionized water. Then solutions of lead ions were added one by one (from 50 ug/mL to 1 mg/mL concentrations) in increasing concentration (from 1 uL of diluted sample to 8 uL of standard lead solution 1 mg/mL), and the scattering at small angles was measured.

The structure analysis was performed by standard procedure according the SAXS method pipeline [35]. The SAXS data was treated in the program suite ATSAS [36], structure parameters such as maximal dimension of the molecule D_{max}, radius of gyration R_g, molecule volume converted to the molecular weight were derived.

RESULTS AND DISCUSSION

SAXS method gives the information about structural parameters of particles randomly placed in solution, such as maximal size, radius of gyration, volume of the particle. The tracking of the changes of aptamer conformation at different external conditions is also important and may be derived from the SAXS data.

Oligonucleotides have mainly the negative charge on their phosphate backbone and one believe that addition of the positively charged ions of Ca^{2+} and Mg^{2+} would play an essential role in the folding of the aptamers. To validate this assumption the different solutions were prepared for the aptamer Gli-233 sample.

SAXS images obtained at the ESRF synchrotron showed that the three-dimensional structure of the Gli-233 doesn't depend on the presence of calcium and magnesium ions for this aptamer as it was assumed before (Fig. 1). Based on the SAXS data processed the D_{max} of the molecule is about 7.0 nm, it has elongated form and most probably contains the double helix fragment in its conformation according to the secondary structure of aptamer sequence. Small-angle scattering yielded the information that Ca^{2+} and Mg^{2+} are not participated in the folding the Gli-233 and maintaining the aptamer spatial structure.

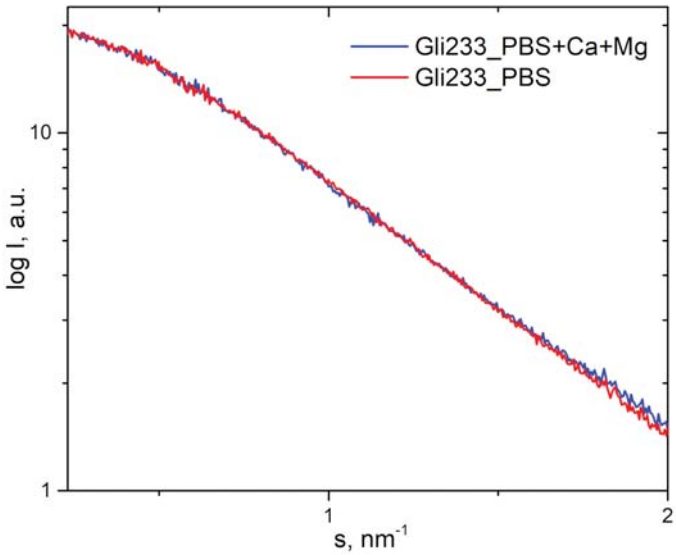
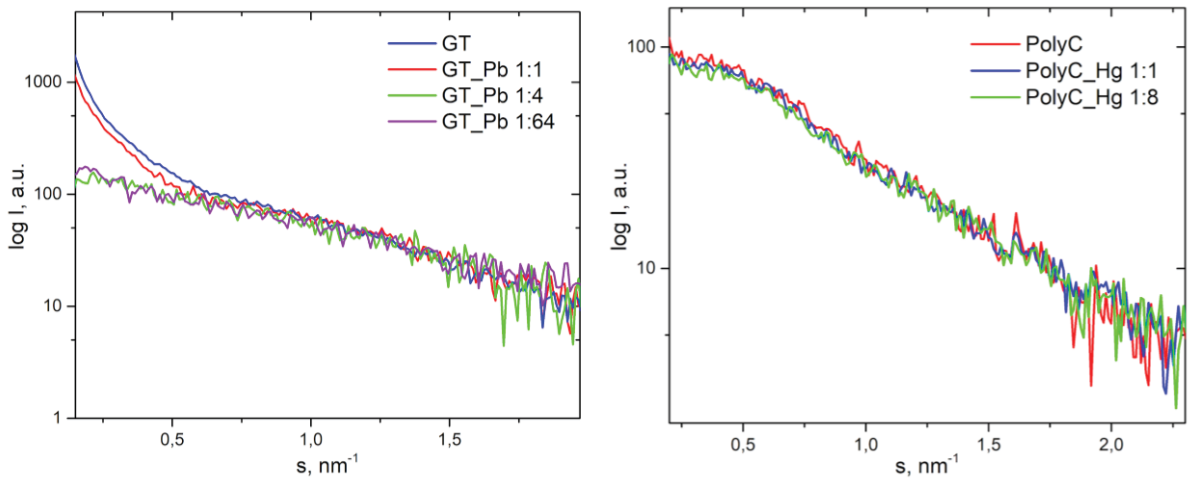


FIGURE 1. SAXS curves for the aptamer Gli-233 in the PBS buffer with addition of Ca^{2+} and Mg^{2+} ions (blue) and without them (red). Coincidence of these curves shows the permanence of the aptamer tertiary structure in both solutions.

For aptamer GT the SAXS data are shown in the range of the scattering vector s from 0.15 to 2 nm^{-1} (Fig. 2a). First two curves (blue and red) correspond to the samples without lead ions and with the one-molar concentration the amount of aptamer respectively. These curves distinctly differ of other two curves which have the many-fold molar ratio of lead in solution respect to the aptamer. High intensity of the scattered X-rays in the range of very small angles ($0.1 < s < 0.5$) implies a probable aggregation of the particles in solution to the large agglomerates.

Further addition of lead to the solution reduces to zero the presence of aggregates. In this case presence of Pb^{2+} ions probably does not change the structure of the aptamer GT molecules (it is suggested from the observation of the same power law for X-ray intensity on the range $0.8 < s < 2.0$ nm^{-1}), but changes their surface charge, that result in breaking down the agglomerates to the separate molecules with addition of metal ions in the solution. It is interesting that a low amount of lead ions is not enough to change the scattering pattern, i.e. some saturation of lead should happen to change the surface charge and make the aptamers to repulse from one another. The form of the scattering data shifts from the possibly aggregated state to the monodisperse solution. This behavior of the molecule structure requires the detailed and comprehensive interpretation utilizing molecular modeling approach.



(a) (b)

FIGURE 2. SAXS results for (a) aptamer GT in the water without Pb^{2+} (blue curve) and after addition of lead into the solution in the GT:Pb molar ratio 1:1 (red), 1:4 (green) and 1:64 (purple); (b) aptamer PolyC in the water without mercury (red curve) and with the molar ratio of PolyC:Hg 1:1 (blue) and 1:8.

Aptamer PolyC shows almost identical SAXS patterns in both cases, without mercury ions in solution and with the presence of them (Fig. 2b). The reason may be in the incorporation of Hg^{2+} ions into the aptamer structure with the negligible weak influence on the aptamer conformation. The most probable conformation of PolyC according to the SAXS data is the open unfolded single-stranded DNA chain due to the aptamer consists of non-complementary nucleotides of one kind.

To reveal accurately the positions of metal ions in the aptamer structure it is needed to apply other complementary methods, for example EXAFS or NMR, in order to localize atoms of the aptamer surrounding the metal atom [37,38].

This study gives an information about conformational changes of DNA aptamers upon binding to the different ions and needs a more detailed consideration of the interaction of different ions with DNA, clarification of the atom positions in the oligonucleotide spatial structure. It may be important for the construction of the biosensors based on the aptamers [39] and the chips for diagnostics [40].

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