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Structure Determination of the Pb^{2+}-specific GT Aptamer Using SAXS and Molecular Modeling Methods

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Abstract. Aptamer GT conjugated with gold nanoparticles is specific for heavy metal ions and can be used for water quality control and environmental monitoring. Circular dichroism (CD) and small-angle X-ray scattering (SAXS) measurements were performed to determine the structure of the aptamer complex with lead (II) ions. Comparison of the measurement results with different options of the spatial structure of the complexes obtained using molecular modeling allowed us to determine how the aptamer changes its conformation due to interacting with the target.

Keywords: aptamers, heavy metal ions, small-angle X-ray scattering, circular dichroism spectroscopy, molecular modeling.

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Introduction

Aptamers are synthetic single-stranded DNA or RNA molecules that are capable of specifically binding to targets of various natures (proteins, viruses, cells, etc.), including heavy metal ions. Aptamer GT is an oligonucleotide rich in guanine and thymine that specifically binds Hg^{2+} and Pb^{2+} ions. [1, 2]. Aptamer GT conjugated with gold nanoparticles can be used for rapid analysis of water for the presence of heavy metal ions. In the presence of target ions, aptamer-coated gold nanoparticles aggregate, resulting in a color change of the solution from pink to blue.

Guanine-rich aptamers are known to interact with lead ions to form G-quadruplex structures [3], where the metal ion occupies the space between the four guanine bases. Depending on the direction of the DNA chain, parallel, antiparallel and mixed quadruplexes are distinguished. In addition, G-quadruplexes can be formed by either one aptamer containing a sufficient amount of guanine or two molecules that retain metal ions [4]. In order to determine the conformation adopted by the GT aptamer upon interaction with the target — Pb^{2+} ions, a combination of SAXS, circular dichroism spectroscopy and molecular modeling methods was used. The combination of these methods made it possible to determine how the structure of the aptamer changes when interacting with the target.

1. Results

1.1. Aptamer in solution without lead ions

In this work, the aptamer GT, which has the sequence 5'-GGGTGGGTGGGTGG-3' and specifically binds $Pb2^{+}$ ions, was investigated [1]. There are no complementary nucleotide pairs in the aptamer, so the structure does not form a duplex part. The GT aptamer is rich in guanines and can form a quadruplex structure in the presence of certain metal ions [5].

In order to study how the structure of the aptamer changes in the presence of the target, at first the SAXS experiment was performed for the GT aptamer in a solution that does not contain heavy metal ions. Based on the obtained data, the following structural parameters of particles formed by the GT aptamer in water were calculated: the maximum dimension $D_{max} = 9.0$ nm and the radius of gyration $R_g = 1.99$ nm. Fig. 1a shows the most probable 3D molecular shape based on the obtained p(r) function according to DAMMIN. Molecular weight of the aptamer in

the solution is 7.3 kDa (in the confidence range 6.7–9.1 kDa) that corresponds to double weight of GT aptamer.

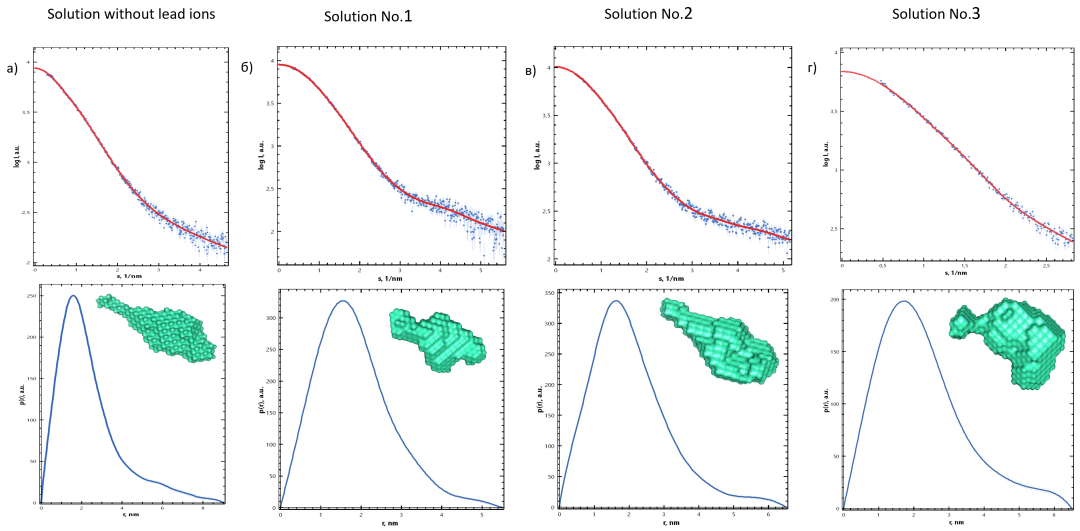

Fig. 1. SAXS data from GT aptamer. Pair distance distribution function p(r) and bead molecular model obtained from the p(r). a) — solution without lead ions; b), c), d) — solutions with different concentration of $Pb^{2}+$ ions according to Tab. 2

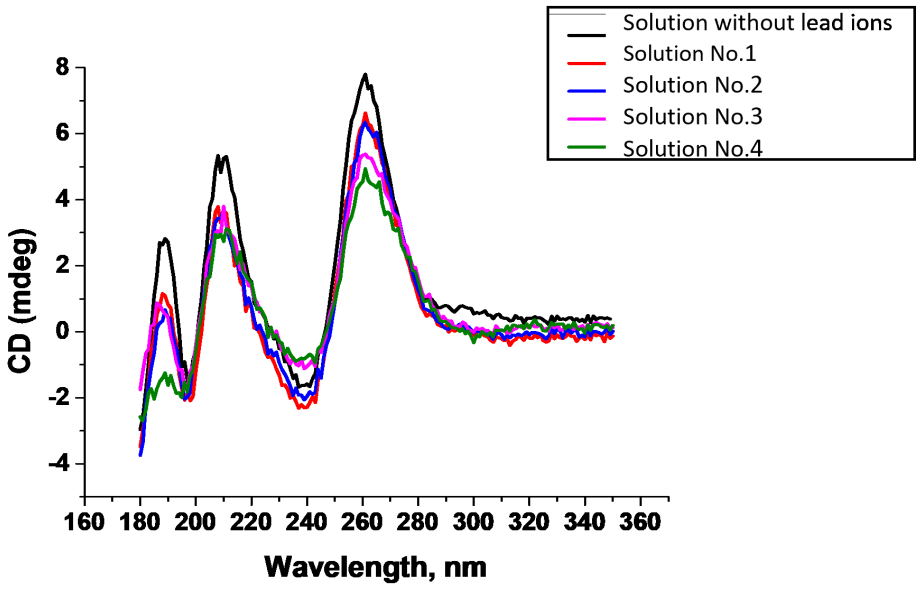

Fig. 2. Circular dichroism spectroscopy data for solutions with different contents of GT aptamer and $Pb^{2}+$ ions

Also, to describe the structure of the GT aptamer, circular dichroism (CD) spectra were obtained in the absence of targets — heavy metal ions (Fig. 2, black curve). The CD spectrum

of the aptamer shows positive maxima characteristic of parallel topology (around 260 nm) [6]. The maxima are found at wavelengths of 211 and 261 nm, the minima at 197 and 241 nm.

To determine the structure of the aptamer in a solution without Pb^{2+} ions, molecular modeling of possible conformations of the GT aptamer was performed: a linear form (Fig. 3a) and a quadruplex-dimer with potassium ions (Fig. 3d). For the aptamer models, molecular dynamics calculations were performed followed by cluster analysis of the obtained trajectories. The values of χ^2 deviations between experimental and simulated SAXS profiles are presented in Tab. 1.

The SAXS and CD data show that even in a solution without Pb^{2+} ions, the aptamer has a parallel quadruplex structure and is in the form of a dimer. However, according to the data in Tab. 1, the χ^2 value for the quadruplex-dimer was high enough to consider this model as uniquely describing the structure of the GT aptamer in solution. Thus, based on the CD, SAXS and modeling data, it can be concluded that the aptamer molecule in the buffer solution is in the form of a quadruplex-dimer, but this is not its only conformation in solution.

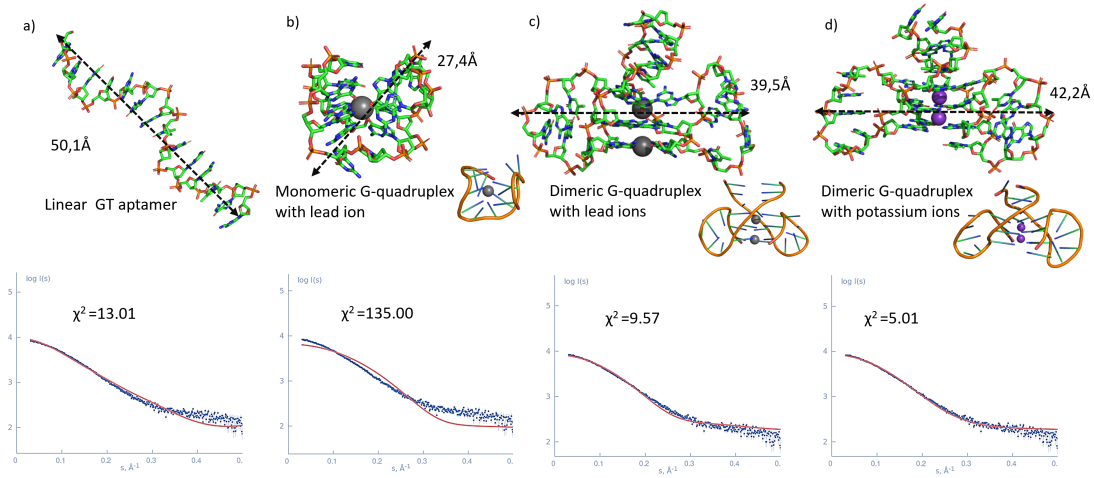

Fig. 3. 3D models of GT aptamer. Comparison of the SAXS model from experiment (blue dashed curve) with structures of GT aptamer (red curve) obtained with MD simulations for Solution No.1. a) linear shape of GT aptamer, b) monomeric quadruplex with Pb^2+ ion, c) dimeric quadruplex with Pb^2+ ions, d) dimeric quadruplex with potassium ions. Carbon atoms are shown in green, nitrogen in blue, phosphorus in orange, oxygen in red, Pb^2+ ions are presented as grey balls, and potassium ions are purple balls. Linear dimensions of the complexes are given in angstroms. Hydrogen atoms are not shown in the figure

1.2. GT aptamer in solutions with different concentrations of Pb^{2+} ions

SAXS experiments were performed for solutions with different concentration ratios of the GT aptamer and Pb^{2+} ions, indicated in Tab. 2. When Pb^{2+} ions are added, the GT aptamer shows signs of a dimeric state for solutions No. 1 and No. 2. In solution No. 3, aggregation of molecules is induced. Solution No. 4 passes into a polydisperse state, for which the SAXS data cannot be interpreted. The SAXS data for solutions No. 1, No. 2, and No. 3 with Pb^{2+} ions are shown in Fig. 1b-d.

When analyzing aptamer solutions with different Pb^{2+} ion contents, CD spectroscopy showed peaks characteristic of parallel quadruplex structures (Fig. 2). Taking into account the CD data,

quadruplex-monomer (Fig. 3b) and quadruplex-dimer (Fig. 3c) models with Pb^{2+} ions were considered. Molecular dynamics calculations showed that both quadruplex models are stable in dynamics. The results of comparison of the complex models with the SAXS results are shown in Fig. 3 (for Solution No. 1) and in Tab. 1. High χ^2 values for the quadruplex-monomer model show that the GT aptamer does not form this type of quadruplex at any ratios of aptamer and metal salt concentrations. In addition to the models containing Pb^{2+} ions, the linear GT aptamer models and the quadruplex-dimer model with potassium ions were compared with the SAXS results, since the aptamer could be present in solution in such forms as well.

Table 1. Comparison of the SAXS model from experiment with structures of GT aptamer obtained with molecular modeling for solutions with different concentrations of Pb^{2+}. Deviations χ^2 were obtained using CRYSOL program. The best fitted models are shown in bold

Solution	Model	χ^2, CRYSOL
Without lead ions	Linear aptamer without metal ions	27,97
Without lead ions	**Dimeric G-quadruplex with K^+ ions**	**12,16**
No.1	Monomeric G-quadruplex with Pb^{2+} ion	135,00
No.1	Dimeric G-quadruplex with Pb^{2+} ions	9,57
No.1	Linear aptamer without metal ions	13,01
№1	**Dimeric G-quadruplex with K^+ ions**	**5,01**
No.2	Monomeric G-quadruplex with Pb^{2+} ion	141,90
№2	**Dimeric G-quadruplex with Pb^{2+} ions**	**11,84**
No.2	Linear aptamer without metal ions	24,54
No.2	Dimeric G-quadruplex with K^+ ions	18,03
No.3	Monomeric G-quadruplex with Pb^{2+} ion	66,04
№3	**Dimeric G-quadruplex with Pb^{2+} ions**	**6,52**
No.3	Linear aptamer without metal ions	12,20
No.3	Dimeric G-quadruplex with K^+ ions	34,06

For solution No 1, the best match with the SAXS results is shown by the quadruplex-dimer model with potassium ions. The quadruplex-dimer model with Pb^{2+} ions has smaller linear dimensions and a more "compact" shape than the similar model with potassium ions (Fig. 3c, 3d). For solution No. 2, the χ^2 value for the dimer with potassium increases significantly (Tab. 1) compared to solution No. 1, which may indicate a rearrangement of structures in the solution [7]. The observed changes in the χ^2 values indicate the replacement of potassium ions by lead (II) ions. Since both potassium and lead form quadruplexes of the same type, and the intensities of the CD peaks for solutions No. 1 and No. 2 are close, it is difficult to estimate the ratio of the number of complexes with different metals. However, in solution No. 3 with an excess of Pb^{2+} ions, judging by the corresponding χ^2 values, we can say that the GT aptamer form in the form of a dimer-quadruplex with Pb^{2+} ions predominate in the solution.

Table 2. The ratio of GT aptamer and Pb^{2+} ions in solutions

Solution	Conc. ions, μg/ml	Added, μl	Ions, ng	Final concentration in sample, μg/ml	Sample volume, μl	Concentration of aptamer in sample, μM
1	50	0.5	25	1.6	15.5	33.3
2	50	1	50	3.13	16.0	32.3
3	50	2	100	5.9	17.0	30.4
4	50	4	200	10.5	19.0	27.2

Comparison of the results of SAXS and CD experiments and molecular modeling shows that the GT aptamer, when interacting with Pb^{2+} ions, forms a structure of a parallel quadruplex formed by two aptamer molecules. Despite the sufficient amount of guanine in the aptamer molecule, the formation of quadruplex monomers is not typical for the GT aptamer.

2. Materials and Methods

2.1. GT aptamer solutions with different concentrations of lead (II)

Oligonucleotide HS-(CH2)6-GGGTGGGTGGGTGG (Apt) was synthesized by Syntol (Moscow, Russia). Aqueous solutions of lead ions with a concentration of 1 g/L were obtained from the Center for Standardization of Samples and Highly Pure Substances (2K-1, St. Petersburg, Russia). Solutions of lead ions with different concentrations were prepared by serial dilution, then added to the aptamer solution.

2.2. Circular dichroism spectroscopy

CD measurements were performed at a constant temperature (20°C) in a thermostatted cell; the drug solution was placed in ultra-thin cuvettes no more than 0.1 mm thick. CD spectra were obtained using a CD dichrograph, model Chiroscan, manufactured by Applied Photophysics (Leatherhead, UK). Measurements were performed in the range of 180–350 nm with 3 repetitions. The aptamer under study and its complex were prepared in water, the aptamer was preheated with a concentration of 250 μM at 95°C for 5 min and cooled to room temperature to restore the conformation.

2.3. Small-angle X-ray scattering

The SAXS measurements for GT aptamer solutions were performed at the BioMUR station in the Kurchatov Institute with synchrotron X-ray radiation at a wavelength of 1.445 $\mathring{A}$ and a sample-detector distance of 500 mm. The GT aptamer solutions were placed in quartz capillaries with a diameter of 1.5 mm and a wall thickness of 0.01 mm. Using a Dectris PILATUS 1M detector, SAXS data were collected within the scattering vector boundaries of $0.1 < s < 6.0$ nm^{-1}. Data processing was performed in the ATSAS software package [8], subtraction of the signal from the buffer solution, data stitching, Guinier and Kratky analysis, calculation of the radius of gyration, molecular volume and molecular mass were performed in the Primus program, the paired distance distribution function was constructed using the AutoGNOM program [9], which is implemented in Primus. Ball-and-stick structures of the total electron density of the molecule

were constructed using the DAMMIN and DAMMIF programs [10]; comparison of theoretical models obtained during molecular modeling was carried out in the CRYSOL program [11].

2.4. Molecular modeling

Modeling of quadruplex structures was performed using the 3D-Nus web server [12]. For the aptamer models, 200 ns molecular dynamics (MD) simulations were performed followed by cluster analysis of the MD trajectories. The MD simulations were performed using the GROMACS software package [13], the Amber14sb force field [14], and the TIP3P water model [15]. The calculations were performed using a periodic cubic box. The box dimensions were determined such that the distance from any aptamer atom to the edge of the box was at least 1.5 nm. Thus, the aptamers in neighboring cells were at a distance of at least 3 nm, which excluded interactions between them. After solvation, the aptamer charge was neutralized with Na+ ions. Molecular dynamics calculations with a duration of 200 ns were performed with an NPT ensemble with a constant number of particles, at a pressure of 1 atm and a temperature of 310 K using a Berendsen thermostat and a Parinello–Raman barostat. Cluster analysis of the obtained molecular dynamics trajectories was performed in the VMD program [16].

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Определение структуры аптамера GT, специфичного к ионам свинца (II), методами МУРР и молекулярного моделирования

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Аннотация. Аптамер GT, конъюгированный с золотыми наночастицами, специфичен к ионам тяжёлых металлов и может использоваться для контроля качества воды и экологического мониторинга. Для определения структуры комплекса аптамера с ионами свинца (II) были проведены измерения кругового дихроизма (КД) и малоуглового рентгеновского рассеяния (МУРР). Сопоставление результатов измерений с разными вариантами пространственной структуры комплексов, полученными с помощью молекулярного моделирования, позволило определить, как меняется конформация аптамера при взаимодействии с мишенью — ионами свинца (II).

Ключевые слова: аптамеры, ионы тяжелых металлов, малоугловое рентгеновское рассеяние, спектроскопия кругового дихроизма, молекулярное моделирование.