ISSN 0027-1314, Moscow University Chemistry Bulletin, 2015, Vol. 70, No. 1, pp. 43–46. © Allerton Press, Inc., 2015.
Original Russian Text © A.V. Yuminova, I.G. Smirnova, A.M. Arutyunyan, A.M. Kopylov, A.V. Golovin, G.V. Pavlova, 2015, published in Vestnik Moskovskogo Universiteta. Khimiya, 2015, No. 1, pp. 51–55.

The Structure of G-Quadruplex Thrombine-binding DNA Aptamer RA36

A. V. Yuminova[a], I. G. Smirnova[b], A. M. Arutyunyan[c], A. M. Kopylov[b], A. V. Golovin[d], and G. V. Pavlova[a]

[a]*OOO Apto-Pharm, Moscow, Russia*
[b]*Department of Chemistry, Moscow State University, Moscow, Russia*
[c]*Belozerskii Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia*
[d]*Department of Bioengineering and Bioinformatics, Moscow State University, Moscow, Russia*
e-mail: sig@genebee.msu.ru

Received October 10, 2014

Abstract—The structure of the 31-meric aptameric DNA oligonucleotide RA36 was studied. This aptamer inhibits the coagulant activity of thrombin more effectively compared with the widely known aptamer 15TGT (thrombin-binding aptamer). RA36 aptamer has a two-pattern structure, which includes two G-rich regions capable of forming a G-quadruplex. We showed by circular dichroism that the aptamer RA36 forms an antiparallel G-quadruplex similar to the G-quadruplex of 15TGT. The thermal stability of G-quadruplex RA36 is significantly lower than that of 15TGT under physiological conditions (concentration of the stabilizing cation 5 mM). The double-quadruplex structure of RA36 is confirmed by the CD spectra of deletion mutants; i.e., G-quadruplex can be formed both by the first and the second G-rich site of aptamer RA36.

Keywords: G-quadruplex, DNA, aptamers, circular dichroism, thermal stability

DOI: 10.3103/S0027131415010095

The new SELEX (Systematic Evolution of Ligands by Exponential Enrichment) technology has emerged as a result of the ability of single-stranded nucleic acids to replicate and form complex tertiary structures [1–5]. The SELEX procedure is used to select small NA molecules known as aptamers, which are the functional analogs of antibodies in terms of their specificity and affinity. Aptamers can form stable complexes with various targets, such as cells, proteins, and low-molecular-weight compounds. Aptamers are used to inhibit enzymes (in particular, proteases).

Thrombin is a multifunctional serine protease involved in the blood coagulation pathway and is the first protease for which aptameric inhibitors have been obtained [6–8].

It was demonstrated by NMR spectroscopy and X-ray diffraction analysis that 15-meric thrombin-binding aptamer dGGTTGGTGTGGTTGG (15 TBA) forms a G-quadruplex [7–10]. Eight guanines form two planar G-quartets with three loops: a TGT loop and two symmetric TT loops.

The CD spectra of antiparallel G-quadruplexes have characteristic positive maxima at 294 and 248 nm and a negative extremum at 265 nm [11–15].

Abbreviations: CD, circular dichroism; PAAG, polyacrylamide gel; 15TGT, a 15-meric thrombine-binding oligonucleotide; RA36, a 31-meric thrombine-binding oligonucleotide; XRD analysis, X-ray diffraction analysis; NA, nucleic acid.

G-quadruplexes are stabilized through tight interaction between cations and guanine residues [16–17]. The cations are coordinated to oxygen atoms (O_6) of carbonyl groups in guanines between the planes of the neighboring G-quadruplexes; the potassium ion is most suitable univalent ion for stabilizing these structures [13].

A systematic study of 15TBA and its derivatives with varied loop length was carried out [14]. It was demonstrated that a parallel G-quadruplex is formed for loops shorter than 5 nucleotides, while antiparallel and mixed quadruplexes are formed in the remaining cases. Chemical modification of the 15TBA structure at certain positions can also result in formation of a parallel G-quadruplex [18].

The maximum (294 nm) and minimum (267 nm) positions in the CD spectrum of 15TBA produced a shift towards shorter wavelengths (to 280 and 252 nm, respectively) in the presence of its complementary DNA strand. This corresponds to the CD spectra of double-stranded DNA [11].

We selected 15- and 31-meric aptamers for this study. The inhibiting activity of RA36 has previously been characterized in terms of its effect on the thrombin time that is used in clinical practice to control thrombin inhibitor therapy [19, 20]. RA36 can be regarded as a two-pattern structure in which both G-rich sites can form a G-quadruplex. However, no data on the structure of aptamer RA36 are currently

Aptameric oligodeoxyribonucleotides under study

Oligodeoxyribonucleotide name	Primary structure, 5'–3'
15TGT	dGGTTGGTGTGGTTGG
RA36	dGGTTGGTGTGGTTGGTGGTTGGTGTGGTTGG
RA36_51	dGTTGGTGTGGTTGGTGGTTGGTGTGGTTGG
RA36_52	dTTGGTGTGGTTGGTGGTTGGTGTGGTTGG
RA36_53	dTGGTGTGGTTGGTGGTTGGTGTGGTTGG
RA36_31	dGGTTGGTGTGGTTGGTGGTTGGTGTGGTTG
RA36_32	dGGTTGGTGTGGTTGGTGGTTGGTGTGGTT
RA36_33	dGGTTGGTGTGGTTGGTGGTTGGTGTGGT
RA36_51_31	dGTTGGTGTGGTTGGTGGTTGGTGTGGTTG

available; hence, this work was aimed at studying the conformational stability of RA36 by circular dichroism.

EXPERIMENTAL CONDITIONS

Materials and Methods. In this study we used synthetic oligodeoxyribonucleotides purified by PAAG (Syntol, Russia). The structures of the oligonucleotides are shown in the table.

The CD spectra were recorded with a CHIRASCAN CD spectrometer (Applied Photophysics Ltd., United Kingdom) and a modified MARK5 dichrograph (Jobin-Yvon, France). The spectra were recorded in the wave-

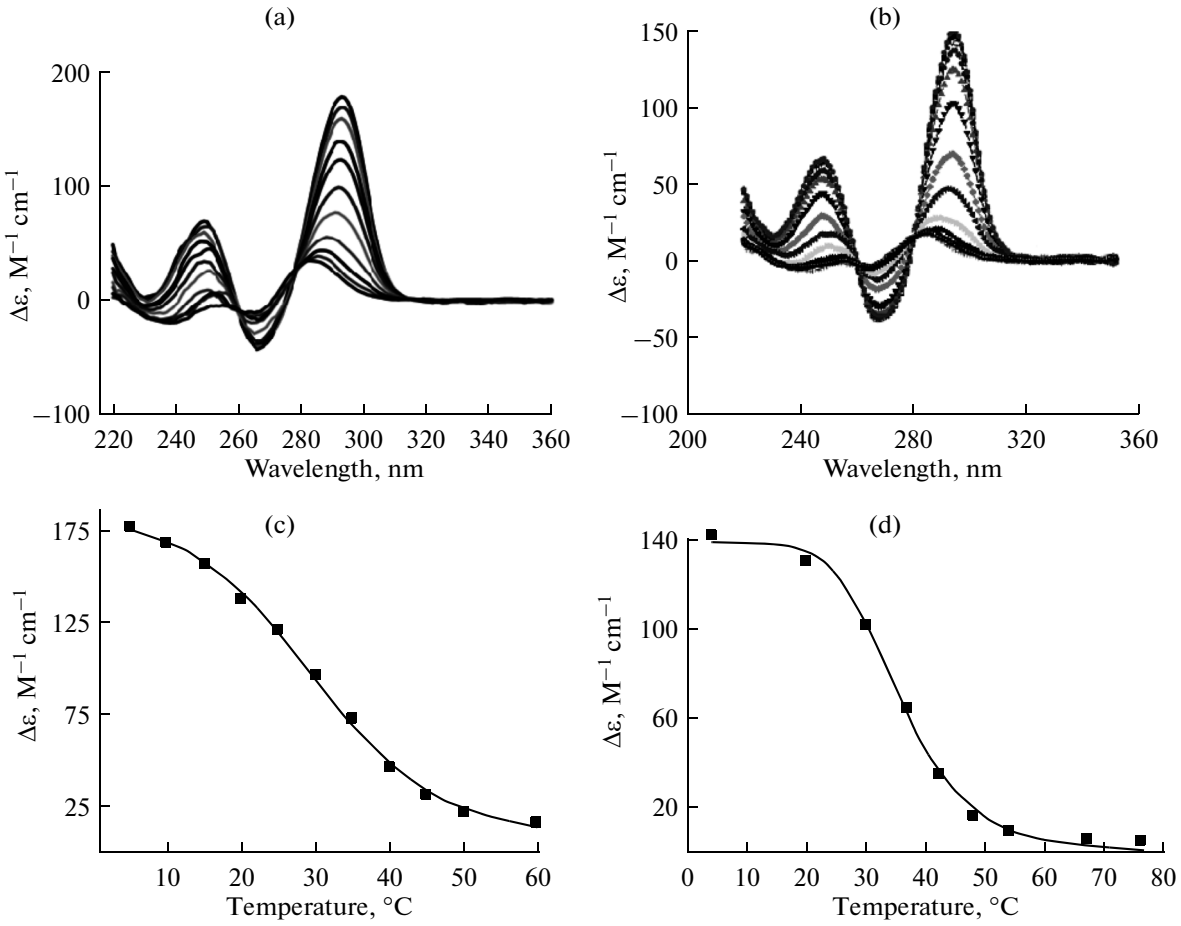

Fig. 1. (a) CD spectra of aptamer RA36 in the temperature range from 5 to 50°C with an increment of 5°C (buffer 20 mM Tris-HCl (pH 7.5), 140 mM NaCl, 5 mM KCl); (b) CD spectra of aptamer 15TGT in the temperature range of 5 to 50°C with an increment of 5°C (buffer 20 mM Tris-HCl (pH 7.5), 140 mM NaCl, 5 mM KCl); (c) thermal stability of the G-quadruplex structure of aptamer RA36 (wavelength 295 nm); (d) thermal stability of the G-quadruplex structure of aptamer 15TGT (wavelength 295 nm).

length range from 220 to 350 nm in a cell with an optical path length of 1 cm at temperatures ranging from 4 to 80°C. Images and mathematical data were processed using JImage, Gimp, GraphWork, and Origin 7.0 software.

RESULTS AND DISCUSSION

Figure 1b shows the CD spectrum of 15TGT, which coincides with the typical CD spectrum of a monomolecular antiparallel G-quadruplex [8–12]. The CD spectrum of RA36 (Fig. 1a) contains maxima at 294 and 247 nm and minima at 267 and 230 nm, which completely correlates with the CD spectrum of 15TGT (Fig. 1b). However, as opposed to 15TGT, the amplitude of the maximum at 294 nm in the CD spectrum of RA36 is higher by 25–35 nm. This effect can be attributed to the formation of two quadruplexes. We studied the structures of a number of RA36 analogs obtained by truncating the RA36 sequence at either 3' or 5' end. Aptamers RA36_51, RA36_52, and RA36_53 produced by the deletion of 1, 2, and 3 nucleotides at the 5' end of the RA36 sequence are also characterized by CD spectra with extrema typical of the antiparallel G-quadruplex. Thus, if there is a degenerated pattern at the 5' end, the second G-quadruplex pattern forms a G-quadruplex (Fig. 2). Figure 3 demonstrates that the melting point of G-quadruplex changes negligibly with decreasing length of the degenerated pattern: 23.5 ± 0.3°C, 24.9 ± 0.2°C, and 24.0 ± 0.9°C for aptamers RA36_51, RA36_52, and RA36_53, respectively.

Aptamers RA36_31, RA36_32, and RA36_33 produced by the deletion of 1, 2, and 3 nucleotides at the 3' end of the RA36 sequence also have the spectra typical of antiparallel G-quadruplex. Thus, if there is a degenerated pattern at the 3' end, the second G-quadruplex pattern also forms a G-quadruplex (Fig. 4). The melting point of G-quadruplex changes negligibly with decreasing length of the degenerated pattern (Fig. 3): 18.1 ± 0.3°C, 18.0 ± 1.0°C, and 17.8 ± 0.5°C for aptamers RA36_31, RA36_32, and RA36_33, respectively. Simultaneous deletion of one nucleotide at the 3' and 5' ends (RA36_31_51), when both patterns are degenerated, eliminates the CD maximum at 294 nm (Fig. 5) characterizing the quadruplex structure. It means that a G-quadruplex was not formed, which correlates with the previously obtained data showing that this DNA oligonucleotide does not exhibit physiological activity [21].

The melting point of aptamer RA36, which was calculated from the changes in amplitude at 294 nm, is much lower than that of 15TGT (30.0 and 39.0°C, respectively), thus indicating that an additional G-rich quadruplex site in RA36 destabilizes the quadruplex structure. It is evident that one of the two quadruplex patterns becomes less stable than 15TGT. Deoxyribonucleotide deletions at the 3' end exhibit a stronger destabilizing effect than deletions at 5' end,

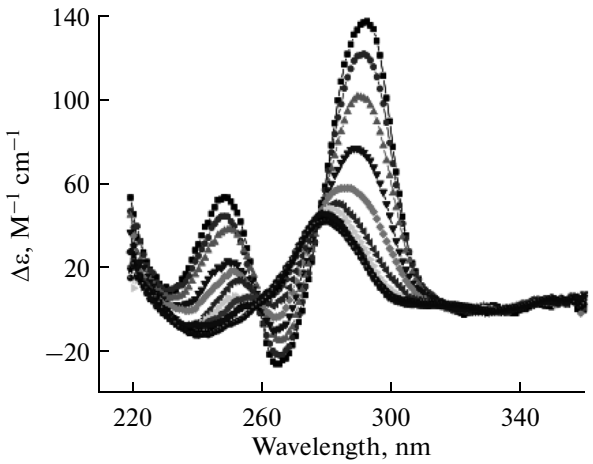

Fig. 2. CD spectra of aptamer RA36_31 in the temperature range from 5 to 50°C with an increment of 5°C (buffer 20 mM Tris-HCl (pH 7.5), 140 mM NaCl, 5 mM KCl).

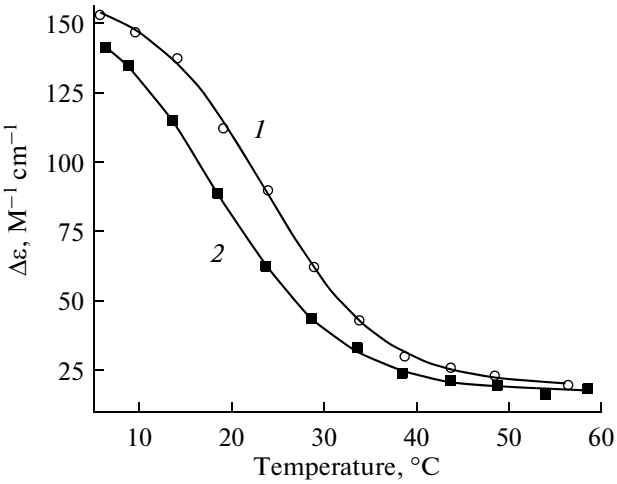

Fig. 3. Thermal stability of the G-quadruplex structure of aptamers (*1*) RA36_31 (T_m = 23.5 ± 0.3°C) and (*2*) RA36_51 (T_m = 18.1 ± 0.3°C), wavelength 295 nm.

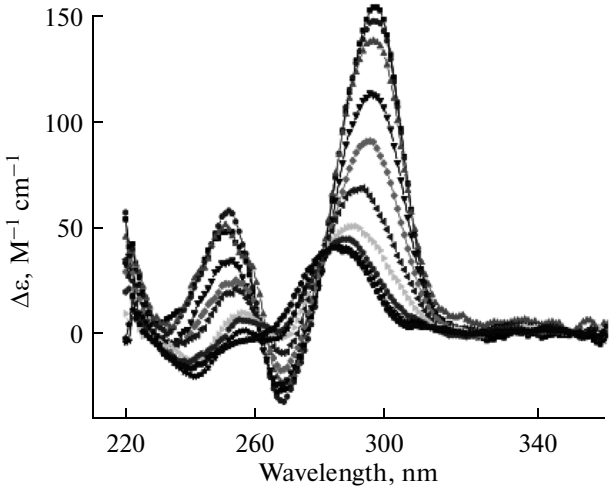

Fig. 4. CD spectra of aptamer RA36_51 in the temperature range from 5 to 50°C with an increment of 5°C (buffer 20 mM Tris-HCl (pH 7.5), 140 mM NaCl, 5 mM KCl).

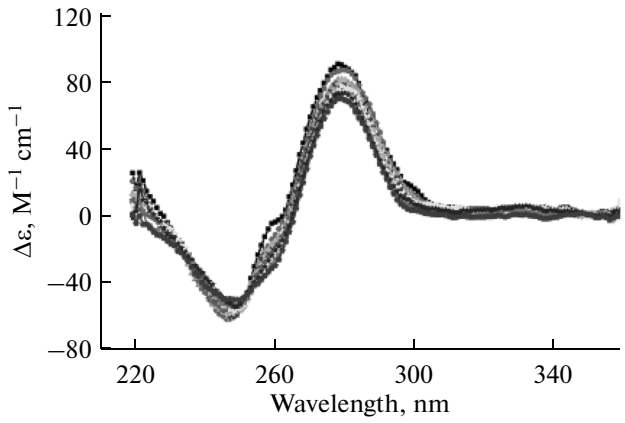

Fig. 5. CD spectra of aptamer RA36_31_51 in the temperature range from 5 to 50°C with an increment of 5°C in buffer 20 mM Tris-HCl (pH 7.5), 140 mM NaCl, 5 mM KCl).

which manifests as a reduction of the melting point of G-quadruplexes (Fig. 5). Deletion analysis gives grounds for suggesting that the quadruplex residing at the 3'-end of RA-36 is more stable than the one residing at the 5' end. It is a curious fact that the structural stability directly correlates with the inhibiting activity of the previously described deletion mutants [21].

Aptamer RA36 forms an antiparallel G-quadruplex that is less stable than the G-quadruplex of the widely known aptamer 15TGT (15TBA). The G-quadruplex can be formed by either the first or the second G-quadruplex pattern of sequence

GTTGGTGTGGTTGGTGGTTGGTGTGGTTGG.

The effect of additional deoxyribonucleotides on G-quadruplex differs for different strand directions: the presence of an additional G-rich sequence at the 5' end exhibits a weaker destabilizing effect on the G-quadruplex of the aptamer compared to that of a similar sequence at the 3' end. In order to effectively design new anticoagulant agents, one needs to understand the causes that determine the structure and properties of thrombin aptamers.

ACKNOWLEDGMENTS

This study was supported by the Russian Foundation for Basic Research (project no. 14-04-01757 a).

REFERENCES

1. Burke, J.M. and Bezzal-Herranz, A., *FASEB J.*, 1993, vol. 7, p. 106.
2. Breaker, R.R. and Joyce, G.F., *TIBTECH Innovations*, 1994, vol. 12, p. 268.
3. Gold, L., Polisky, B., Uhlenbeck, O., and Yarus, M., *Annu. Rev. Biochem.*, 1995, vol. 64, p. 763.
4. Osborne, S.E., Matsumura, I., and Ellington, A.D., *Curr. Opin. Chem. Biol.*, 1997, vol. 1, p. 5.
5. Kopylov, A.M. and Spiridonova, V.A., *Mol. Biol.* (Moscow), 2000, vol. 34, no. 6, pp. 940–954.
6. Bock, L.C., Griffin, L.C., Latham, J.A., Vermaas, E.H., and Toole, J.J., *Nature*, 1992, vol. 355, p. 564.
7. Tasset, D.M., Kubik, M.F., and Steiner, W., *J. Mol. Biol.*, 1997, vol. 272, p. 688.
8. Macaya, R.F., Schultze, P., Smith, F.W., Roe, J. A., and Feiqon, J., *Proc. Natl. Acad. Sci. U.S.A.*, 1993, vol. 90, p. 3745.
9. Padmanabhang, K., Padmanabhang, K.P., Ferrarag, J.D., Sadled, J.E., and Tulinsky, A., *J. Biol. Chem.*, 1993, vol. 268, p. 17651.
10. Padmanabhang, K. and Tulinsky, A., *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 1996, vol. 52, p. 272.
11. Berova, N., Nakanishi, K., and Woody, R.W., *Circular Dichroism: Principles and Applications*, Weinheim, 2000, p. 724.
12. Kumar, N. and Maiti, S., *Biochem. Biophys. Res. Commun.,* 2004, vol. 319, p. 759.
13. Kankia, B.I. and Marky, L.A., *J. Am. Chem. Soc.*, 2001, vol. 123, p. 10799.
14. Smirnov, I.V. and Shafer, R.H., *J. Mol. Biol.*, 2000, vol. 296, p. 1.
15. Smirnov, I.V. and Shafer, R.H., *Biochemistry*, 2000, vol. 39, p. 1462.
16. Chen, F.-M., *Biochemistry*, 1992, vol. 31, p. 3769.
17. Basu, S., Szewczak, A.A., Cocco, M., and Strobel, S.A., *J. Am. Chem. Soc.*, 2000, vol. 122, p. 3240.
18. Peng, C.G. and Damha, M.J., *Nucleic Acids Res.*, 2007, vol. 35, no. 15, p. 4977.
19. Savchik, E.Y., Kalinina, T.B., Drozd, N.N., Makarov, V.A., Zav'yalova, E.G., Lapsheva, E.N., Mudrik, N.N., Babij, A.V., Pavlova, G.V., Golovin, A.V., and Kopylov, A.M., *Bull. Exp. Biol. Med.*, 2013, vol. 156, no. 1, p. 44.
20. Golovin, A.V., Reshetnikov, R.A., Zavyalova, E.G., Kopylov, A.M., Pavlova, G.V., and Babij, A.V., RF Patent 2429293, 2009.
21. Zavyalova, E., Golovin, A., Pavlova, G., and Kopylov, A., Module-activity relationship of G-quadruplex based DNA aptamers for human thrombin, *Curr. Med. Chem.*, 2013, vol. 20, no. 38, p. 4836.

Translated by D. Terpilovskaya