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A comparative thermodynamic study for both natural and artificial RNA/DNA–protein binary complexes

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Abstract

In recent years, Systematic Evolution of Ligands by EXponential enrichment (SELEX) technique has been developed into a fast growing field. In contrast to traditional recognition elements, like antibody, our interests focus on novel molecular recognition elements based on nucleic acids, which are of value both for the therapy and biosensors. A comparative study of thermodynamic for both natural and artificial RNA/DNA–protein complexes would establish bases for a specificity of complex formation. In particular, we have shown that aptamers could be used for a direct measuring of thrombin enzymatic activity in a solution. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: RNA/DNA–protein interactions; Ribosome; SELEX; RNA/DNA aptamers; Thrombin; Enzymatic activity

1. Introduction

Wide-scale molecule testing requires a development of fast and easy-to-use devices, like biosensor. The devices intimately couple a biological recognition element, interacting with a target analyte, with a physical transducer that translated the bio-recognition event into a useful signal.

In contrast to traditional recognition elements, like antibody, our interests focus on novel molecular recognition elements based on nucleic acids, which are of value for biosensors. The unique network structure of single-stranded DNA and RNA provides a ground for affinity and specificity; and recognition is a basic principle of DNA/RNA–protein interactions. The parameters of these interactions could be described in terms of thermodynamic, i.e. dissociation constant (K_d). A comparative study of thermodynamic for both natural and artificial RNA/DNA-protein complexes would establish bases for a specificity of complex formation.

Aptamer is a single-stranded oligonucleotide that could specifically bind with high affinity and specificity to a selected target molecule, for example drug, protein, etc. Aptamers are discovered by in vitro selection process known as Systematic Evolution of Ligands by EXponential enrichment (SELEX) [1,2]. Generally, SELEX-derived aptamers could be considered as functional analogues of monoclonal antibodies. A huge library of single-stranded oligonucleotides, differing in nucleotide sequence, represents an array of differently shaped molecules with different affinities for a different areas of a given target protein. An initial template is obtained by automatic chemical synthesis of DNA fragments, and each has a random sequence of 30–60 nt. Several rounds of selection yield a fraction enriched in aptamers, with relative binding several orders of magnitude higher compared to the initial library. Finally, an aptamer pool is cloned, individual aptamers are sequenced, and their affinity for a ligand estimated, providing data for choosing the best aptamers (''winners'').

This paper describes a power of DNA aptamer to be used for a direct measurement of thrombin enzymatic activity in solution.

2. Experimental

Aptamer 5′-CAGTCCGTGGTAGGGCAGGTTGGGGTGACT-3′ was synthesized on an Applied Biosystems 380B oligonucleotide synthesizer by phosphoroamidate method.

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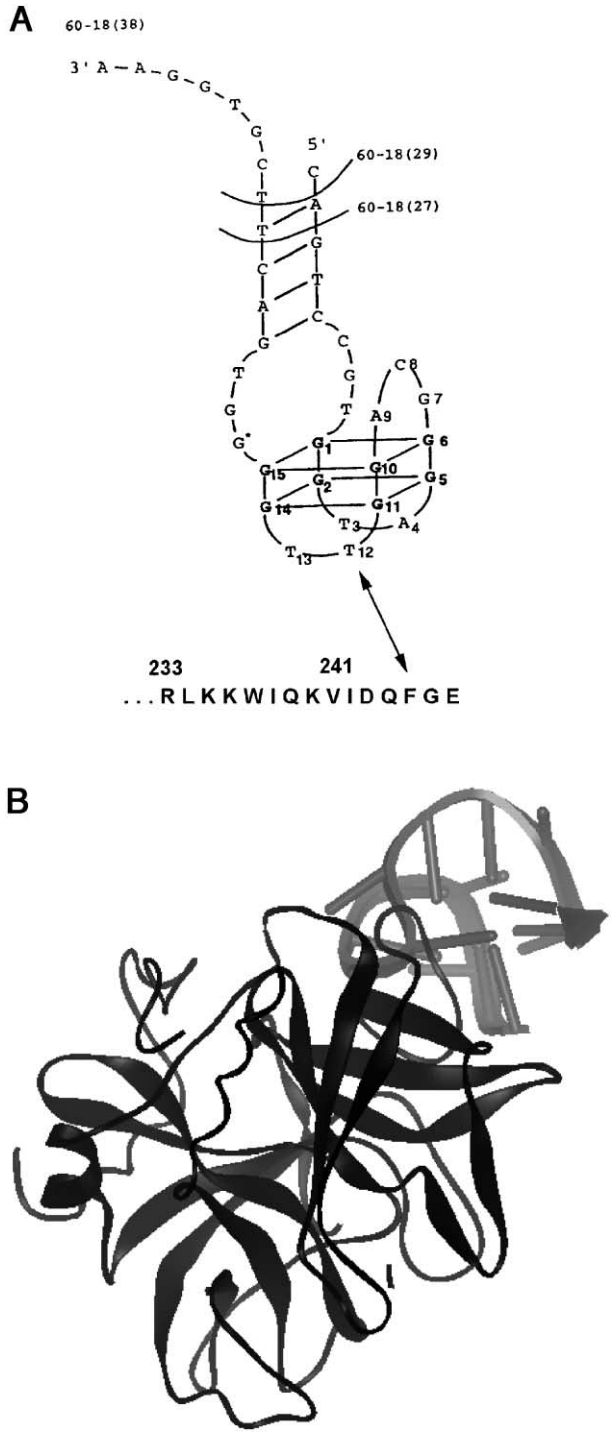

Fig. 1. A secondary structure of DNA aptamer (A), and a tertiary structure model of the aptamer–thrombin complex (B) [8].

Human α-thrombin "Fluka" (Switzerland) had 600 NIH U/mg, α-human thrombin "Renam" (Russia) had 4500 NIH U/mg. Binding assays were carried out by nitrocellulose filter partitioning [8]. Buffer was 20 mM Tris–Ac pH 7.4, 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$. The dissociation constant is defined as the protein concentration at a half value of isotherm flattening, provided that protein concentration is far greater than that of titrated DNA aptamer.

3. Results and discussion

As an example of natural RNA–protein interaction study, the complexes of prokaryotic ribosomal protein S7 with different RNA has been chosen. In vitro EcoS7 is able to bind to both *Escherichia coli* 16S rRNA fragment and *E. coli* S12–S7 intercistronic region. It allows to study a property of a single protein to recognize two different RNA, by measuring apparent dissociation constants (K_d) by nitrocellulose (NC) filter-binding assay. The EcoS7–16S rRNA fragment complex has K_d=6.5±1.7 nM. TthS7 is able

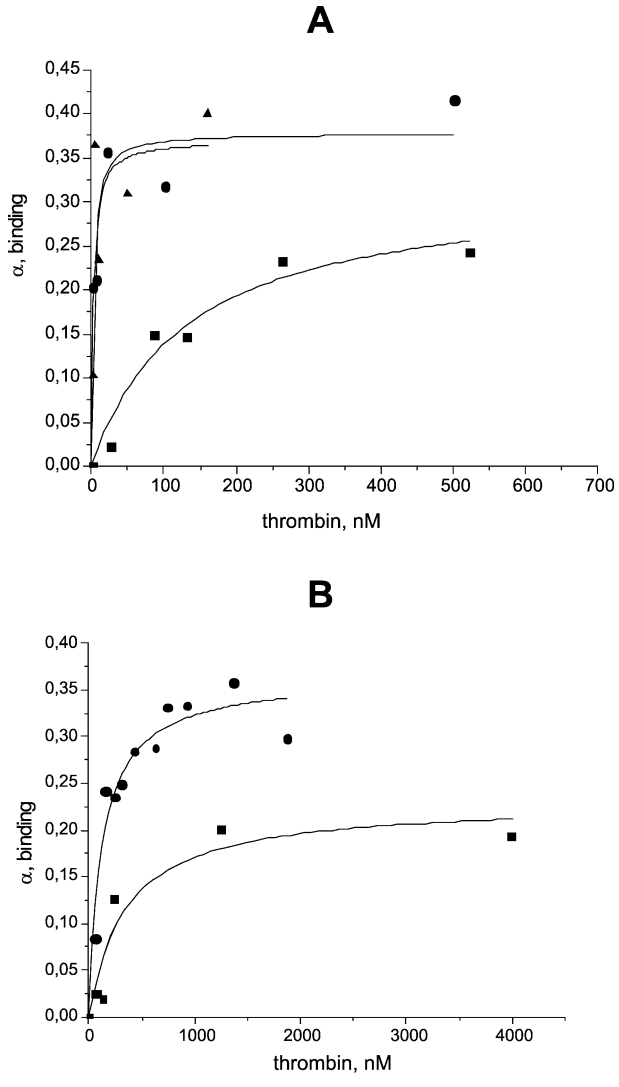

Fig. 2. Isotherms of thrombin binding to the DNA aptamer under different conditions. Complex formation was done at 37 °C for 15 min. Circles and triangles graph—thrombin activity 4500 NIH U/mg, squares graph—thrombin activity 600 NIH U/mg. 20 mM Tris–Ac, pH 7,6; 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1mM $CaCl_2$. 20 mM Tris–Ac, pH 7,6; 100 mM NaCl, 1 mM $MgCl_2$.

to bind to Eco16S rRNA with K_d=36±6 nM, which is high enough for a heterologous complex.

SELEX technique, applied to S12–S7 intercistronic fragment (67 ns) of *str* mRNA [3], has yielded aptamers to TthS7 with nucleotide sequences different from the natural variant. The aptamer has K_d=50±8 nM, which is very close to the value of heterologous complex. The results of the structural–thermodynamic study of the natural RNA–protein complexes provide a solid base to extend the approach to artificial complexes.

Thrombin is a multifunctional serine protease with both pro-coagulant and anti-coagulant functions, therefore has a high medical significance [4].

The number of authors selected the different families of DNA aptamers for thrombin [5–8]. One of the best aptamers is 5′ -CAGTCCGTGGTAGGGCAGGTTGGGGTGACT-3′ , which forms two G-quartets with an additional DNA duplex (Fig. 1) The aptamer binds to the protein with K_d=0.5 nM [8]. Fig. 2A shows the aptamer binding isotherms for thrombin, obtained by NC filter binding assay. The buffer has the same salt components as blood: 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$. Two different samples of thrombin have been used: from "Fluka"—600 NIH U/mg, and from "Renam" (Russia)—4.500 NIH U/mg. It turned out that DNA aptamer binding to thrombin with high enzymatic specific activity is 20 times higher than Fluka thrombin. Therefore, it has been clearly demonstrated that the values of K_d depend on enzymatic activity of thrombin and not on total protein concentration. The same correlation was found for some different buffer conditions (Fig. 2B), despite the absence of K^+, which stimulates G-quartet assembly of the aptamer.

Therefore, the DNA aptamer could be used for a direct measuring of thrombin enzymatic activity in solution.

When the manuscript has been already applied we have learned that Lee and Walt [9] have applied DNA aptamer for fiber-optic biosensor for thrombin.

Acknowledgements

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