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Ensemble and single-molecule biophysical characterization of D17.4 DNA aptamer–IgE interactions☆

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ABSTRACT

Background: The IgE-binding DNA aptamer 17.4 is known to inhibit the interaction of IgE with the high-affinity IgE Fc receptor FcεRI. While this and other aptamers have been widely used and studied, there has been relatively little investigation of the kinetics and energetics of their interactions with their targets, by either single-molecule or ensemble methods.

Methods: The dissociation kinetics of the D17.4/IgE complex and the effects of temperature and ionic strength were studied using fluorescence anisotropy and single-molecule spectroscopy, and activation parameters calculated.

Results: The dissociation of D17.4/IgE complex showed a strong dependence on temperature and salt concentration. The k_{off} of D17.4/IgE complex was calculated to be $(2.92 \pm 0.18) \times 10^{-3}$ s^{-1} at 50 mM NaCl, and $(1.44 \pm 0.02) \times 10^{-2}$ s^{-1} at 300 mM NaCl, both in 1 mM $MgCl_2$ and 25 °C. The dissociation activation energy for the D17.4/IgE complex, E_a, was 16.0 ± 1.9 kcal mol^{-1} at 50 mM NaCl and 1 mM $MgCl_2$. Interestingly, we found that the C19A mutant of D17.4 with stabilized stem structure showed slower dissociation kinetics compared to D17.4. Single-molecule observations of surface-immobilized D17.4/IgE showed much faster dissociation kinetics, and heterogeneity not observable by ensemble techniques.

Conclusions: The increasing k_{off} value with increasing salt concentration is attributed to the electrostatic interactions between D17.4/IgE. We found that both the changes in activation enthalpy and activation entropy are insignificant with increasing NaCl concentration. The slower dissociation of the mutant C19A/IgE complex is likely due to the enhanced stability of the aptamer.

General significance: The activation parameters obtained by applying transition state analysis to kinetic data can provide details on mechanisms of molecular recognition and have applications in drug design. Single-molecule dissociation kinetics showed greater kinetic complexity than was observed in the ensemble in-solution systems, potentially reflecting conformational heterogeneity of the aptamer. This article is part of a Special Issue entitled: Physiological Enzymology and Protein Functions.

1. Introduction

Aptamers are library-selected single-stranded oligonucleotide (DNA or RNA) ligands that fold into three-dimensional structures and are capable of binding to pre-selected targets. Aptamers usually are selected using SELEX (Systematic Evolution of Ligands by EXponential enrichment), an in vitro process involving iterative binding, elution, and PCR amplification [1,2], which allows isolation of target-specific DNA or RNA molecules from a large pool (~10^{15}) of random oligonucleotides. The first aptamer-based therapeutic for wet, age-related macular degeneration was approved by FDA in 2005 [3] and aptamers for treating various other diseases (e.g., acute coronary syndrome, von Willebrand factor related disorders, angiomas, acute myeloid leukemia) are currently in clinical trials [4] with two of them in phase 3 [5]. The anti-thrombin aptamer [6] that acts as an anti-coagulant agent has been used as a short-term anti-coagulant designed for the application in the coronary artery bypass graft surgery and its optimized form is under phase 2 clinical trials with the added advantage that complimentary oligonucleotides efficiently reverse the activity of the aptamer

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providing a method for regulating the action of the therapeutic [7]. Apart from therapeutics, aptamers are being considered for a wide range of other applications including diagnostics, forensics, and biodefense [8–10].

IgE has been demonstrated to play a major role in mammalian immune defense by eliciting protective responses including local inflammation, itching, and mucus production [11]. A crucial step in these responses is the binding of IgE antibodies to the IgE Fc receptor (FcεRI), found primarily on mast cells and basophils [12]. One promising approach to treatment of IgE-mediated diseases is to block IgE/FcεRI interactions [13,14]. Omalizumab, an FDA-approved humanized anti-IgE monoclonal antibody that targets and binds to circulating free IgE and prevents the interaction of IgE with the FcεRI receptor, is used for treating severe persistent allergic asthma including manifestations of exposure to peanuts in patients with peanut allergy [15]. Wiegand and co-workers [12] reported a SELEX-isolated IgE-binding DNA aptamer, D17.4, that was shown to inhibit the binding of IgE to FcεRI. The binding site of D17.4 is reported to be identical with or at least in close proximity to the Cε3 region contact point of IgE with FcεRI [12]. The D17.4 aptamer has been extensively studied, and found to have a low-nanomolar affinity for IgE by various methodologies (10 nM using filter binding [12], 15 ± 4 nM using fluorescence anisotropy [16], 8 nM using capillary electrophoresis [17]). The D17.4 aptamer has been used as a recognition element in biosensing for sensitive IgE detection [18–23]. The mfold-predicted [24] stem-loop structure of D17.4 at $[Na^+] = 138$ mM, $[Mg^{2+}] = 1$ mM and 25 °C is shown in Fig. 1A.

Most prior work on aptamer/protein interactions has focused on characterizing binding affinity, identifying residues involved in aptamer recognition, and application of aptamers in therapeutics [4,25] and biosensing [18–21]; relatively little is known about the kinetics and energetics of aptamer/protein interactions. In this work we studied the dissociation kinetics, the effects of temperature and ionic strength, and the kinetic activation parameters of the D17.4 aptamer/IgE complex using fluorescence anisotropy [26–30]. From the dissociation kinetic data at various temperatures we calculated the dissociation activation energy (E_a), activation enthalpy ($\Delta H^{\#}$), and activation entropy ($\Delta S^{\#}$) for the D17.4 aptamer/IgE complex. The role of specific structural features of the D17.4 aptamer was investigated using single-nucleotide mutations.

The study of dynamic biological events at the nanoscale has been greatly advanced in recent years by the advent of single molecule spectroscopy (SMS). Recent advances in SMS have made it possible to discern individual dynamical events within complex biological processes such as binding [31–34] and structural fluctuations [35–38]. Single molecule imaging by Total Internal Reflectance Fluorescence (TIRF) microscopy and fluorescence resonance energy transfer are useful in measuring single association/dissociation events without the ensemble averaging that occurs in bulk measurements [39–44]. By measuring time-dependent signals over large areas, it is possible to extract equilibrium and non-equilibrium kinetic values for many molecules in parallel, directly from single molecule dwell time analysis, and characterize distributions of functionality and behavior (e.g., aptamers [45,46]). Here we use SMS to observe the heterogeneity of the dissociation kinetics of IgE from the D17.4 aptamer under variable ionic strength.

2. Materials and methods

2.1. Reagents

The 37-mer IgE-binding aptamer (D17.4), with the sequence 5′-GGGGCACGTTTATCCGTCCCTCCTAGTGGCGTGCCCC-3′, was used as reported in the literature [12]. HPLC-purified (unlabeled, 5′-labeled with Texas Red and 3′-modified with biotin (separated from the DNA sequence with a T_{15} spacer) with or without a 5′-Cy5 fluorophore) D17.4 aptamer was purchased from Integrated DNA Technologies (IDT, Coralville, IA, USA). Texas Red-labeled mutant aptamers bearing a single-nucleotide change in the original D17.4 sequence, D17.4-G1T (G at position 1 was replaced with T), D17.4-C19A and D17.4-C19G, also were from IDT. Human IgE protein (from plasma) was purchased from Athens Research and Technology Inc. (Athens, GA, USA; 16-16-090705-M) and used without further purification. Vectabond (aminosilane; SP1800) was purchased from Vector Laboratories (Burlingame, CA, USA). Methoxy-PEG-NHS (MW: 5,000; 85969-1G) and biotin-PEG-NHS (MW: 5,000; BI-050TS) were purchased from Sigma-Aldrich (St. Louis,

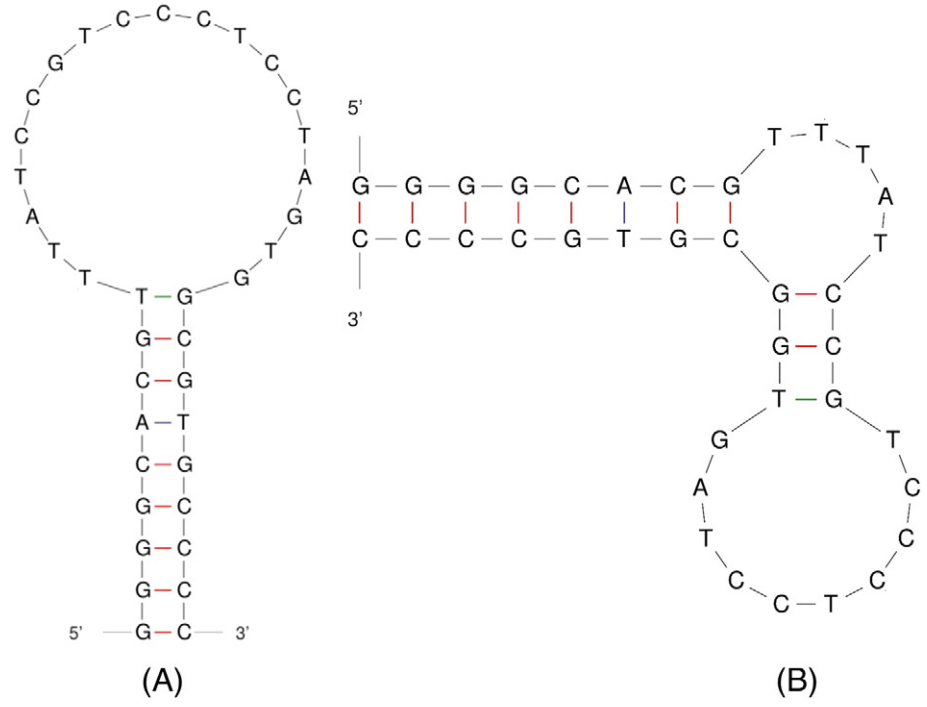

Fig. 1. Mfold-predicted secondary structure of D17.4 aptamer; (A) at $[Na^+] = 138$ mM, $[Mg^{2+}] = 1$ mM and 25 °C, $\Delta G = -9.93$ kcal/mol; predicted secondary structure remains the same at $[Na^+] = 50$ mM ($\Delta G = -9.53$ kcal/mol) or 200 mM ($\Delta G = -10.15$ kcal/mol) and $[Mg^{2+}] = 1$ mM. (B) at $[Na^+] = 300$ mM, $[Mg^{2+}] = 1$ mM and 25 °C, $\Delta G = -9.40$ kcal/mol.