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Characterisation of charge distribution and stability of aptamer-thrombin binding interaction

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ABSTRACT

Aptamers are single stranded nucleic acids with specific target-binding functionalities, biophysical and biochemical properties. The binding performance of aptamers to their cognate targets is influenced by the physicochemical conditions of the binding system particularly in relation to biomolecular charge distribution and hydrodynamic conformations in solution. Herein, we report the use of zeta potential measurements to characterise the surface charge distribution, biomolecular hydrodynamic size and the binding performance of a 15-mer thrombin binding aptamer (TBA) to thrombin under various physicochemical conditions of pH, temperature, monovalent (K^+) and divalent (Mg^{2+}) cation concentrations. Charge distribution analysis demonstrated time dependence in the formation of stable TBA-thrombin and TBA-thrombin-metal ion complexes. TBA was characterised to be most stable in pH above 9. The presence of monovalent and divalent metal ions reduced the electronegativity of TBA through electrostatic interactions, and this demonstrated to improve binding characteristics. TBA-thrombin complexes generated under different physicochemical conditions showed varying surface charge distributions. The stability of TBA-thrombin complex investigated using Scatchard analysis showed that the presence of K^+ increased the binding performance by displaying a positive cooperativity relationship. The presence of Mg^{2+} showed a concave upward trend, potentially caused by heterogeneity in binding.

1. Introduction

Aptamers are single-stranded DNA/RNA ligands generated by means of an iterative process known as Systematic Evolution of Ligands by Exponential enrichment (SELEX) [1–3]. They are a preferred class of bioprobes owing to their stability, non-immunogenicity, low cost of generation, wide spectrum of target space, minimal ethical issues, and the ability to undergo pre-/post biomodification processes [4,5]. Notwithstanding, the binding performance of aptamers to their target molecules is a function of the physicochemical conditions of the binding system including pH, presence of metal ions, temperature and chemomolecular labelling/modifications of the aptamer. This affects the kinetics of the binding process, charge and conformational distributions of the aptamer and the stability of the complex. An in-depth biophysical characterisation of aptamer-target interactions is critical to improve understanding of the binding process in order to establish optimal binding conditions for diverse applications.

The 15-mer thrombin binding aptamer (TBA) [5′-GGT TGG TGT GGT TGG-3′] is commonly used as a model aptamer to characterise aptamer-target interactions [6–9]. Biophysical techniques such as circular dichroism, isothermal titration and surface plasmon resonance have been used to investigate the effects of binding conditions such as the presence of metal ions and variations in thrombin concentration on the binding characteristics of 15-mer and 29-mer TBA. Lin and coworkers reported that 15-mer TBA binds to exosite I (fibrinogen recognition site) of thrombin by electrostatic interaction. They also reported that the presence of K^+ induces the formation of G-quadruplex conformations of TBA [7]. By using affinity capillary electrophoresis, Girardot, Gareil and Varenne [10] studied the effect of monovalent (Na^+, K^+, Cs^+) and divalent (Mg^{2+}, Ca^{2+}, Ba^{2+}) metal ions on the binding interactions of a lysozyme aptamer. A mobility swift was observed during the addition of cations to the electropheretic medium, potentially due to the interactions between aptamer molecules and cations and further inducing a conformational change of the aptamer. Girardot, Li, Descroix and Varenne [11] employed a microchip electrophoresis technique in a frontal mode to characterise the binding behaviour of a lysozyme aptamer under different conditions of ionic strength, divalent cations and thermal effect. The results showed that

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the presence of divalent metal ions improved binding affinity. Also thermal treatment of the aptamer increased conformational stability which further resulted in improved binding affinity.

In addition, the electrokinetics and binding traits of aptamers change with varying conditions of the binding medium [7,12,13]. Zeta (ζ) potential analysis via dynamic light scattering is a useful technique in characterising interfacial distribution and electrokinetic behaviour of charged species in aqueous media [14,15], and would advance theoretical understanding of the binding behaviour of aptamers in relation to surface charge distribution, hydrodynamic mobility and binding characteristics in solution. Despite the uniqueness of ζ potential as a biophysical parameter for biomolecular characterisation and complex formation, there is limited research focused on the application of ζ potential analysis to investigate the binding characteristics of aptamers to their cognate target molecules and the stability of the emerging biomolecular complex. This study focuses on the application of ζ potential analysis as a tool to investigate the effect of varying surface charge distribution and hydrodynamic mobility on aptamer binding characteristics, and the stability of the aptamer-target complex. It contributes to building a robust theoretical understanding that underpins the binding behaviours of aptamers, providing basis to optimise binding performance for a wide range of applications including biosensor, bioseparation, cell targeting, high throughput screening, in vitro evolution, and drug delivery.

2. Materials and methods

2.1. Biomolecules and reagents

15-mer thrombin aptamer 5′-GGT TGG TGT GGT TGG-3′ was purchased from First Base (Malaysia). Thrombin and Arsenazo III were purchased from Sigma-Aldrich (Malaysia). Potassium Chloride, Magnesium Chloride, Calcium Chloride and Tris-EDTA (TE) buffer were obtained from Fisher Scientific (Malaysia).

2.2. Instrumentation

Nanoplus particulate system (Nanoplus-3, USA) was used to perform zeta potential and average hydrodynamic size measurements of TBA, thrombin and thrombin-TBA complex at 25 °C. Zeta potential analysis was performed with forward scattering optics at 15° and a voltage of 60 V. Hydrodynamic size measurement was performed with Backscatter Optics at 160°. All measurements were taken after the equilibrium time of one minute. Measurements for each analysis were triplicated, and all aqueous media were formulated with Milli-Q water purification system. An illustration of the principle of dynamic light scattering technique in characterising the zeta potential of TBA and thrombin-TBA complex is shown in Fig. 1.

2.3. Zeta potential analysis of TBA and thrombin-TBA complex

2.3.1. Effect of pH

100 μM aptamer solutions were diluted in pH-adjusted TE buffers ranging from pH 4–11 to a final volume of 1 mL and incubated for 30 min at 25 °C. Zeta potential measurements were performed for each pH system. To study the effect of pH on TBA-thrombin complex, 100 μM aptamer solutions were interacted with 7.5 mg/mL thrombin solution and pH-adjusted TE buffer in a volumetric ratio of 1:1:2 to make a final volume of 1 mL. The mixtures were incubated for 30 min at 25 °C. For zeta size analysis, 1600 μM TBA aliquots were diluted in pH-adjusted TE buffers ranging from pH 4–11 and incubated for 30 min at 25 °C. 1600 μM TBA aliquots were interacted with 7.5 mg/mL thrombin solution and pH-adjusted TE buffer in 1:1:1 volumetric ratio, followed by incubation for 30 min at 25 °C. Zeta size measurement was performed for each pH system.

2.3.2. Effect of metal ion polarity and concentrations

The effect of different metal ions on the surface charge distribution of TBA was investigated. Two different metal ions Magnesium (Mg^{2+}) and Potassium (K^{+}) with concentrations 0.01 M, 0.1 M, 0.5 M, 1.0 M and 1.5 M were prepared and interacted with 500 uL of TBA or Thrombin-TBA complex. The resulting solutions were then incubated for 30 min at 25 °C followed by zeta potential measurements. For zeta size measurement, TBA (1600 μM) and thrombin-TBA complex (7.5 mg/mL for thrombin) were measured after interaction with varying concentrations of metal ions in incubation for 30 min at 25 °C.

2.3.3. Effect of temperature

100 μM aptamer solution was diluted with TE at pH 8, made up to a final volume of 1 mL, and incubated for 30 min under varying temperature conditions of 20 °C, 25 °C, 30 °C, 37 °C and 45 °C. Thrombin-TBA complex was prepared by mixing 100 μM aptamer solution with 7.5 mg/mL thrombin solution, followed by incubation at the above-mentioned temperatures. To understand the impact of temperature on the hydrodynamic size and zeta potential of the complex, 1600 μM aptamer solution was reacted with thrombin (7.5 mg/mL) at a volumetric ratio of 1:1 and incubated at the different temperatures.

2.4. Scatchard analysis

Scatchard analysis was performed to investigate the binding of metal ions to TBA and Thrombin-TBA complex. Arsenazo III was used to bind free metal ions K^{+} and Mg^{2+}. Arsenazo stock solution of 250 μM was prepared with 20 mL of the prepared solution aliquoted and diluted to 50 μM. This was followed by adjustment of the pH to 9 by the addition of NaOH. 20 μL of 10 μM TBA was incubated with 20 μL of metal ions with varying concentrations 0.01, 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 1.5 and 2.0 M for 30 min. 10 μL aliquots were served into a 96 well flat bottom plate, and 250 μL of 50 μM Arsenazo was added to each well with gentle mixing. The system was allowed to incubate for 10 min before taking absorbance measurements using a microplate spectrophotometer at 610 nm wavelength. An analogous experiment was carried out using TBA-thrombin complex. The concentration of free/unbound cations in solution was determined from the absorbance readings using a standard curve. A Scatchard plots of bound/free cation concentrations versus TBA (Thrombin-TBA complex) bound cation concentrations were developed.

3. Results and discussion

3.1. Effect of pH on the characteristics of TBA and thrombin-TBA complex

As different agents for biomarking and stabilisation may present non-isocratic conditions that can affect the binding affinity and chemical properties of aptamers, the effect of pH from 4 to 11 was investigated. Zeta potential analysis of TBA surface charge distribution demonstrated electronegativity over the range of pH as shown in Fig. 2(A). This was primarily due to the presence of negatively charged phosphate groups on the base DNA aptamer sequence. Also, the structure of G-quadruplex with rich guanine and centered by carbonyl oxygen increases the electronegative stability of TBA. The results indicated that the surface charge of the aptamer increased in electronegativity with increasing pH. The zeta potential of the aptamer was slightly positive at pH 4, with an isoelectric focusing (pI) point of ~4.1. The increase in aptamer electronegativity under increasing pH was as a result of deprotonation associated with increasing concentration of hydroxyl groups exposed to the aptamer. Also, high hydroxyl concentrations can result in structural conformational changes to induce redistribution of negatively charged moieties of the aptamer molecule. The positive zeta potential observed for pH < pI was due to the presence of high concentration of H^{+} ions (protonation) that neutralise localised negative charges on the aptamer molecule. The aptamer was