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(REVIEW ARTICLE)

Spectroscopy of the thrombin-aptamer interaction: A review

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Abstract

Thrombin is considered an adaptable enzyme, and it has a major role in the process of injured vessel hemostasis. Hence, the detection of thrombin is crucial in the course of any illness. Aptamers, a class related to functional molecules, are short artificial oligonucleotides that are being widely used in protein sensors as recognition elements. Tracking and detection of different biomolecules is crucial not just in the medical diagnostics field but in biotechnology and biological research, biodefense, drug discovery, drug design, and environmental monitoring as well. Direct detection methods, including surface plasmon resonance spectroscopy and mass spectrometry, are also extensively utilized for the detection of thrombin-aptamer interactions. We summarize the studies on spectroscopy and the thrombin-aptamer interaction in this review.

Keywords: Thrombin; Aptamers; Spectroscopy; Hemostasis

1. Introduction

Thrombin is considered an adaptable enzyme, and it has a major role in the mechanism of injured vessel hemostasis [1, 2]. In addition, sufficient research has recommended that thrombin, acting as a protease activator of many receptors, exerts its effects on the development and occurrence of various vascular disorders, Alzheimer's disease, and cancer cell migration [3–5].

Consequently, the exact ascertainment of the content of thrombin is beneficial in determining the course of illness and developing the therapy regimen. Recently, there have been some assay techniques to determine the development of clots in medical practices, including thrombin time (TT), partial thromboplastin time (PTT), and prothrombin time (PT). Nevertheless, the above-mentioned techniques can only contemplate the general adequacy of blood clotting and are not applicable to the thrombin content alone. Hence, to attain quantitation for thrombin, several identification techniques, such as the neotype method, require to be addressed.

Aptamers, a class related to functional molecules, are short artificial oligonucleotides (RNA or DNA) in an approximate range of 10–100 molecules containing a phosphate and a nucleoside, which have particularly significant tendencies toward different targets, including adenosine triphosphate (ATP) [6], proteins [7], and amino acids [8]. They are being widely used in protein sensors as recognition elements [9].

Aptamers for certain targets can be easily detached from oligonucleotide combinatorial libraries by the ligands' systematic molecular evolution via the exponential enrichment (SELEX) method [10]. In comparison to antibodies, aptamers possess high tendencies for target binding as well. Additionally, the low penetrability and immunogenicity of tissue allow aptamers to be utilized in specific designations where antibodies might not be attained. Because of their

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many benefits, including modification, specificity, high stability, convenient synthesis, and affinity, aptamers are developing into significant substitutes for several frequently utilized antibodies, as might be ratified by their use in the identification of cancer markers, metallic ions, biotoxins, and different hazardous compounds [11–20]. The use of aptamers' specific recognition capability in conjunction with conventional techniques and nanomaterials significantly improved the action of thrombin detection [21-23]. In this review, we will summarize the literature related to thrombin binding aptamers (TBA) and spectroscopy for thrombin identification.

2. Thrombin Binding Aptamer (TBA)

A nucleic acid aptamer was reported by Bock et al., for the first time ever, that coheres to human thrombin [24]. Following that, a diverse set of thrombin functional aptamers was identified [25-31]. TBA1 and TBA2 are some of the most frequently used aptamers among them. TBA1 is regarded as a DNA aptamer (50 GGTTGGTGTGGTTGG-30) cohering to the exosite for fibrinogen-recognition of human thrombin, while TBA2 is also regarded as a DNA aptamer (50 AGTCCGTGGTAGGGCAGGTTGGGGTGACT-30) cohering to the exosite for heparin-binding of human thrombin with greater affinity. Thrombin aptamers can fold to form G-quadruplexes during the recognition process, which may cohere hemin to form hemin/G-quadruplex DNAzymes. Wu et al. created an electrochemical thrombin-sensor by conjugating TBA1 to a short peptide. A platform for assay was developed by Li et al., in reference to the optical characteristics of gold nanoparticles, also known as AuNPs, that were perceived simply but effectively for thrombin detection with extremely high sensitivity. Using TBA1 (the detection probe) and TBA2 (the capture probe) simultaneously, Liu et al. developed an electrochemical sensor related to click chemistry for the efficient and uncomplicated detection of thrombin, which had significant practical applications [32, 33]. The action of the molecular layer developed by the probe molecule present on the sensor surface can also alter the sensitivity [34]. A study conducted by Ma et al. determined the viability of electrostatic modulation subsequent to the interaction and binding between aptamer and thrombin and also indicated an underlying use [35].

3. Detection of aptamer-thrombin attachment – spectroscopy

Tracking and detecting different biomolecules is crucial not just in the clinical diagnostics field but also in biotechnology and biological research, biodefense, drug development, and environmental monitoring. Researchers from various fields have worked together to develop approaches and methods that are simple, economical, precise, vigorous, and high throughput. Recently, most methods utilized for the identification of biomolecules that require molecular recognition episodes (such as the formation of aptamer-analyte complexes) are deviations from enzyme-linked immunosorbent assays (a type of sandwich ELISA) and generally depend on the utilization of receptors tagged along with probe molecules, which might not be perpetually advantageous. [36-38].

Biomolecular labelling is a challenging procedure that can result in the decline of biological activity. Various assays utilize nanoparticle tendencies to develop aggregates, which leads to a change in colour because of the deviation in the surface plasmon resonance in reaction to binding episodes. Although this is regarded as a convenient procedure, it is restricted by its sensitivity to environmental changes, including alteration in pH and ionic strength, which can lead to colour change and aggregation. Another nanoparticle-related method takes advantage of the metal nanoparticles' ability to extinguish emission from adjacent fluorescent substances. In that instance, the analyte's existence is marked by the alteration in the emission intensity from the fluorophores that cohere to the receptor [39]. The identification of such analytes is critical for disease treatment and screening, as well as environmental monitoring [40].

Direct detection methods, including SPR (surface plasmon resonance spectroscopy) and mass spectroscopy, are extensively utilized as well; however, these methods are costly and probably restricted by extensive preparation of the sample. For instance, mass spectrometry needs purification and isolation of the sample (generally via chromatography). Surface plasmon resonance, which marks molecular interaction by estimating the alteration in the index of refraction at the metal surface functionalized with a conjugated receptor, is based on nonspecific interconnections [39]. Ma et al. employed force spectroscopy to explore the interaction between aptamer and its targets under specific physiological circumstances. Both MD simulation and DFS experiments were utilized during this study to explore thrombin-aptamer binding. MD simulation can also be used to explore the mechanisms related to surface stress change. The results showed that the dissociation force of the aptamer-target complex is less than the forces linked with the G-quadruplex breakdown [41, 42].

In a study, Li et al. created a sensitive and simple electrochemical impedance spectroscopy (EIS) biosensor based on an aptamer-cohering thrombin as a molecular identification element for thrombin determination. The study demonstrates that gold nanoparticles (GNPs) electrodeposited on a glassy carbon electrode (GCE) and utilized as a thiolated aptamer

immobilisation platform can boost EIS biosensor sensitivity for protein determination. This study also determines that the EIS technique is a competent technique for the ascertainment of both dissociation and association constants on GCE [43].

4. Conclusion

Spectroscopy can be beneficial for the determination of medical biomarkers, threat agents (chemical or biological), as well as environmental pollutants. It can also be utilised as a more specific and precise method for thrombin-aptamer complex detection.

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