Biomaterials and Biosensors

Review Article

Advances in rapid detection of Pseudomonas aeruginosa with DNase-based sensors

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ABSTRACT

Pseudomonas aeruginosa is a common pathogen, and its presence in medical environments and water bodies has attracted widespread attention. Traditional detection methods are usually time-consuming and cumbersome, so it is necessary to develop a rapid and sensitive detection technology. DNase can specifically recognize and cut DNA molecules complementary to its substrate sequence. The researchers took advantage of this property to design various DNase-based sensors for detecting the presence of Pseudomonas aeruginosa. These sensors usually use DNase as a recognition element to identify target strains by hybridizing with specific DNA sequences. When the target strain is present, DNase is activated and begins to catalyze the cleavage reaction, producing a detectable signal. This DNase-based sensor has the advantages of rapidity, high sensitivity, and high specificity. In addition, the researchers also explored combining DNase with nanomaterials, fluorescent dyes, etc. to further improve the performance of the sensor. These improvements have improved the detection ability of the sensor in complex samples, laying the foundation for practical applications. With the continuous improvement of technology, these sensors are expected to be widely used in medical, environmental monitoring and other fields, and provide more efficient and convenient solutions for bacterial detection. This study reviewed the research progress of DNase-based sensors for the rapid detection of Pseudomonas aeruginosa.

KEYWORDS

DNase; Sensor; Pseudomonas aeruginosa

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1. Introduction

Pseudomonas aeruginosa, a widespread gram-negative pathogen, inhabits diverse natural environments and is a significant agent of hospital-acquired infections [1]. Its menace to human health is underscored by its capacity for multi-drug resistance and the formation of resilient biofilms. Prompt and precise detection of Pseudomonas aeruginosa is pivotal for timely infection control and treatment. Conventional detection methods for this pathogen are notorious for their time-consuming protocols and intricate procedures [2,3]. Thus, the imperative to devise swift, sensitive, and dependable detection methods, especially for clinical and public health applications, becomes evident.

DNA enzyme-based sensors have gained extensive traction as a novel detection technology in recent years. These sensors harness DNA enzymes' unique recognition and binding prowess to swiftly identify Pseudomonas aeruginosa [4-7]. Under specific conditions, DNA enzymes can specifically bind to DNA and catalyze reactions. This binding specificity renders DNase an ideal biological recognition molecule, enabling the construction of highly specific sensors. Prior investigations have highlighted significant strides in rapidly detecting Pseudomonas aeruginosa through DNA enzyme-based sensors [8, 9]. These sensor designs primarily leverage the DNA enzyme's capability to selectively bind to Pseudomonas aeruginosa, with the detection of complex formation or signal alterations signifying the presence of the pathogen. Currently, DNA enzymes like DNase I and DNase II are widely employed in Pseudomonas aeruginosa detection. By coupling them with fluorescent probes or other detection methods, efficient and swift identification of Pseudomonas aeruginosa has been realized [10, 11].

However, challenges persist in the DNA enzyme-based sensor paradigm. Environmental conditions demand meticulous consideration, and mitigating interference from potential substances necessitates further enhancement and optimization. Thus, future research can be directed toward refining the sensor's sensitivity, specificity, and stability to better align with clinical demands. This review consolidates the research advancements in DNA enzyme-based sensors for rapidly detecting Pseudomonas aeruginosa.

2. Hazards of Pseudomonas aeruginosa and Detection Imperatives

Pseudomonas aeruginosa, a commonplace gram-negative bacterium, thrives in natural habitats like soil, water, and plant surfaces. While it plays a pivotal ecological role, it also poses a notable risk to human health-especially among those with compromised immune systems and hospital patients [13-15]. Infections stemming from Pseudomonas aeruginosa wield grave consequences, encompassing skin, respiratory, urinary tract, blood, and traumatic infections. Individuals with diminished immunity, such as immunosuppressed and critically burned patients, confront heightened perils, potentially leading to severe complications and fatality. The pathogen's multi-drug resistance is a paramount challenge, undermining treatment and control. It employs various resistance mechanisms, including β-lactamase production, multi-drug efflux pumps, and biofilm formation. These mechanisms defy conventional antibiotics, amplifying the complexity of infections and treatment challenges. Swift and precise Pseudomonas aeruginosa detection is indispensable for timely intervention. However, conventional methods-like bacterial culture and fluorescent antibody staining-entail prolonged protocols and harbor the risk of erroneous results.

There's a pressing demand for a rapid, sensitive, and reliable detection approach. This method should possess robust specificity, differentiating Pseudomonas aeruginosa from other bacteria and delivering swift and accurate infection determinations [16]. Additionally, it should exhibit high sensitivity, enabling dependable outcomes even with low bacterial loads. The ideal scenario involves a simplified, expedient, and user-friendly procedure adaptable to clinical, hospital, and laboratory contexts. DNA enzyme-based sensors emerge as a novel detection avenue with the potential to expedite Pseudomonas aeruginosa detection. By harnessing DNase's binding specificity and catalytic activity with target molecules, these sensors hold promise for swift and accurate identification of the

pathogen. The development and application of DNA enzyme sensors could substantially enhance the efficiency and precision of Pseudomonas aeruginosa detection, effectively meeting the challenge posed by this formidable infection.

3. Working Principle and Advantages of DNA Enzyme Sensors

DNA enzyme sensors represent a class of sensors that exploit DNA enzymes' distinctive binding and catalytic capabilities to achieve detection. Their operational principle is rooted in the specific binding and catalytic degradation interplay between DNase and DNA substrates. The initial step of a DNA enzyme sensor involves the association of DNA enzyme with target molecules, like Pseudomonas aeruginosa, generating complexes via precise binding. This binding typically occurs in the target molecule's DNA through the interaction of DNase with a specific recognition sequence or structure [17-19]. DNase exhibits pronounced specificity, selectively binding to the DNA of the target molecule while disregarding non-target molecules. Once DNase engages the DNA of the target molecule, the sensor progresses to the catalytic degradation stage. DNA enzymes can recognize and bind DNA substrates, eliciting catalytic activity under specific conditions. DNA substrates often bind specifically to DNase or DNA molecules harboring distinct sequences in sensor applications. DNase-DNA substrate binding catalyzes substrate degradation, resulting in DNA strand fragmentation, sequence cleavage, or other chemical changes. This catalytic degradation prompts observable signal alterations, such as enhanced fluorescence, color shifts, or modifications in electrochemical signals. The final phase of a DNA enzyme sensor involves detecting the signal change and correlating it with the presence of the target molecule. Diverse detection methods can be employed based on the sensor's design. Common techniques encompass fluorescence detection, absorbance measurement, and electrochemical detection. Fluorescence detection, frequently utilized in DNA enzyme sensors, incorporates fluorescent probes. The binding of DNA enzyme to the target molecule, catalyzing the degradation reaction, leads to fluorescence signal changes, such as augmentation or quenching. Another prevalent method is absorbance measurement, wherein the DNA substrate in the sensor forms a complex with dyes. DNase-driven substrate degradation modifies the dye's absorbance, enabling signal change detection through spectral measurements [20]. Electrochemical detection hinges on alterations in electrochemical signals to ascertain the target molecule's presence. The sensor's DNA substrate is usually combined with an electrochemical label. DNase-mediated substrate degradation results in electrochemical signal modifications-such as current or potential fluctuations-that can be identified via electrochemical techniques. The foundational principle of the DNA enzyme sensor is to expedite the precise detection of target molecules by capitalizing on the DNA enzyme's specific binding and catalytic degradation. These sensors demonstrate remarkable sensitivity and specificity, rendering them suitable for many fields, including medical diagnostics, environmental monitoring, and food safety.

DNA enzyme sensors offer numerous advantages, rendering them an ideal molecular detection tool. They exhibit high sensitivity and specific catalytic activity, capable of detecting exceedingly low concentrations of target molecules and amplifying signals, thereby enhancing detection sensitivity and specificity. These sensors excel in curbing non-specific binding, reducing the risk of false alarms and misdiagnoses, and elevating detection accuracy. Furthermore, DNA enzyme sensors permit real-time monitoring, facilitating swift and accurate ongoing detection to meet the demands of rapid response and real-time surveillance. Their user-friendly operation and simplicity circumvent the need for intricate sample processing, accommodating operators of varying skill levels. Programmability empowers the customization and optimization of sensors to meet the diverse requirements of different target molecules. Their multifunctionality extends to various applications such as quantitative analysis, qualitative identification, and sequence determination [21]. In summary, DNA enzyme sensors' advantages encompass heightened sensitivity, specific catalytic activity, non-specific binding elimination, real-time monitoring, simplicity, programmability, and versatility. These attributes position DNA enzyme sensors for widespread

application across medicine, environment, food safety, and beyond, presenting a robust solution for rapid and accurate molecular detection.

4. Current Methods for Detecting Pseudomonas aeruginosa Using DNA Enzyme Sensors

DNA enzyme-based sensors have been extensively explored and employed for detecting Pseudomonas aeruginosa. These methodologies leverage DNase's specific binding and catalytic capabilities to swiftly and precisely identify the pathogen. The DNA enzyme-fluorescent probe method employs fluorescent probes that bind to both the DNA target of the DNA enzyme and Pseudomonas aeruginosa. The fluorescent probe is released upon DNase binding and catalyzing the target DNA's degradation, leading to heightened fluorescence signals. The presence of Pseudomonas aeruginosa can be determined by gauging changes in fluorescence intensity. Alternatively, the DNA enzyme-electrochemical approach involves DNA enzyme binding to target DNA and initiating a degradation reaction, yielding alterations in the electrochemical signal. This typically employs DNA substrates with electrochemical markers. As DNase degrades the substrate, changes in the markers' current or potential are detected through electrochemical methods, effectively detecting Pseudomonas aeruginosa. Another method, the DNA enzyme-fluorescent dye approach, sees DNA enzyme binding to target DNA and catalyzing its degradation, leading to the dissociation of DNA bound with fluorescent dye, inducing changes in fluorescence signals [22]. Detecting shifts in fluorescence intensity promptly reveals the presence of Pseudomonas aeruginosa. The DNA enzyme-nanomaterial method amalgamates DNA enzymes with nanomaterials to form a complex. When this complex binds to target DNA and catalyzes degradation, the properties of the nanomaterials change, resulting in heightened fluorescence signals or alterations in absorption spectra. Detecting these changes facilitates the swift detection of Pseudomonas aeruginosa. These DNA enzyme-based sensor methods consistently demonstrate robust sensitivity, specificity, and rapidity in detecting Pseudomonas aeruginosa. However, challenges persist, necessitating further sensitivity, specificity, experimental conditions, cost-efficiency, and operational simplicity enhancements. As technology advances, the methods for detecting Pseudomonas aeruginosa based on DNA enzyme sensors are poised for refinements and developments, expanding the repertoire of rapid and accurate detection options.

4.1. DNase I Sensor

A DNase I sensor is a sensor that exploits the interaction between DNase I (deoxynuclease I) and target molecules to achieve detection. DNase I is an enzyme known for its capability to catalyze DNA degradation. In the context of a DNase I sensor, detection can pertain to either the presence of DNA or the extent of DNA degradation, thus enabling the identification of specific molecules or cells. The initial step of a DNase I sensor involves binding the target molecule with DNase I. This interaction is achieved by pairing a specific target sequence or structure with DNase I. The target molecule could encompass DNA, RNA, or other molecules possessing a specific binding affinity to DNase I. Once DNase I binds to the target molecule, the sensor progresses to the subsequent stage-DNase I catalyzed degradation. DNase I initiates the cleavage of DNA or RNA chains within the target molecule, leading to their fragmentation and consequent degradation. The final phase of the DNase I sensor revolves around detecting signal changes resulting from the degradation reaction, which can then be correlated with the presence of the target molecules.

Various detection methods can be applied to capture these signal changes, including fluorescence detection, absorbance measurement, and electrochemical detection [23]. Fluorescence detection ranks among the frequently employed methods in DNase I sensors. This involves combining fluorescent dyes with target molecules. When DNase I catalyzes the degradation of these target molecules, the intensity of fluorescence signals experiences

change, such as fluorescence enhancement or quenching. Analyzing the alteration in fluorescence signals enables the determination of target molecules' existence or degree of degradation. Another commonly used method is absorbance measurement, where target molecules within the sensor form complexes with dyes. As DNase I catalyzes the degradation of these target molecules, the absorbance of the dyes undergoes modification. This signal change can be detected through spectral measurements. Electrochemical detection, on the other hand, relies on shifts in electrochemical signals to indicate the presence of target molecules. The sensor's target molecules are typically conjugated with electrochemical labels. DNase I catalyzed degradation of these target molecules leads to changes in electrochemical signals (such as current or potential), which are detectable through electrochemical techniques. The DNase I sensor harnesses the interaction between DNase I and target molecules to rapidly and accurately detect specific molecules or cells. This sensor exhibits notable specificity and sensitivity, rendering it a promising tool for biomedical research, clinical diagnostics, and environmental monitoring applications. (Fig.1)

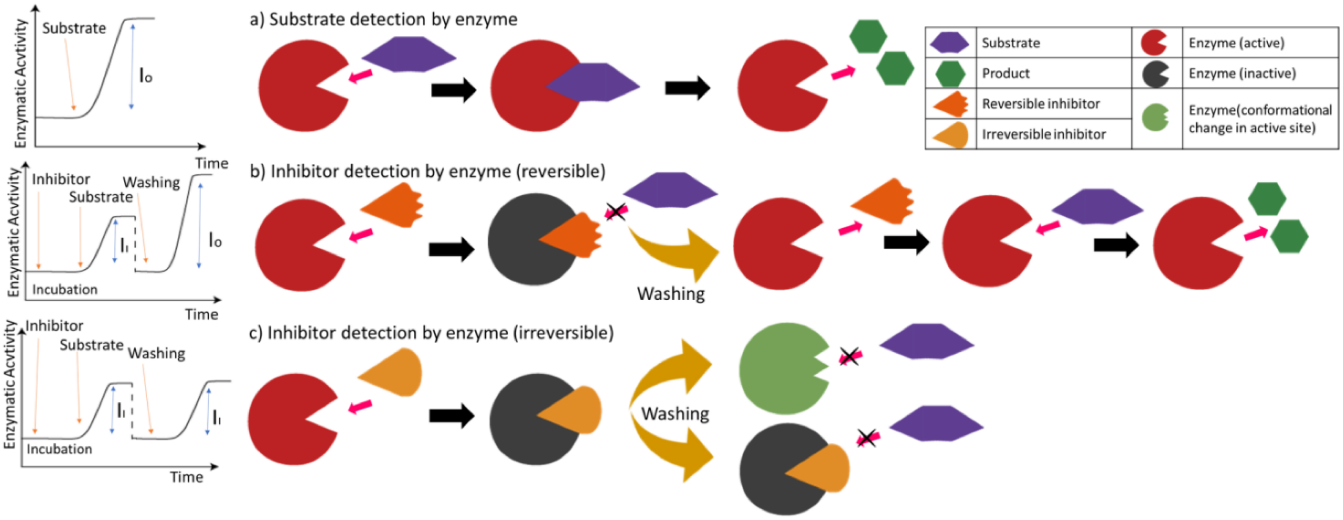

Figure 1. Scheme of enzymatic biosensor for substrate and inhibitor (reversible and irreversible) detection.

Note: Enzymatic biosensors can incorporate electrochemical, optical, and calorimetric transducers. Among these transducers, electrochemical ones are the type most commonly used in literature, probably due to the first developed biosensor being an enzymatic electrochemical biosensor (Clark). (Sensors (Basel) 2018,18(6):1924.)

4.2. DNase II Sensor

To facilitate detection, the DNase II sensor is built upon the interaction between DNase II (deoxynuclease II) and target molecules. DNase II is an enzyme recognized for its role in catalyzing DNA degradation within lysosomes. Similar to the DNase I sensor, the DNase II sensor enables the detection of DNA's presence or the extent of its degradation, thus enabling the identification of specific molecules or cells. The initial step of the DNase II sensor parallels that of the DNase I sensor-it involves binding the target molecule with DNase II. This interaction is achieved by pairing a target sequence or structure with DNase II. Similar to DNase I, DNase II's target molecule repertoire encompasses DNA, RNA, or other molecules with specific binding sites for DNase II [24]. Once DNase II binds to the target molecule, the sensor proceeds to the catalytic degradation phase. DNase II initiates the DNA degradation within the target molecule, breaking it down into smaller fragments and resulting in its degradation.

The final phase of the DNase II sensor mirrors the previous sensors, focusing on detecting the signal changes caused by the degradation reaction and correlating them with the presence of target molecules. Diverse detection methods, such as fluorescence detection, absorbance measurement, and electrochemical detection, can be employed. Fluorescence detection, as before, is commonly used in DNase II sensors. Fluorescent dyes are combined with target molecules. Upon DNase II catalyzed degradation of target molecules, the fluorescence signal intensity

undergoes alteration, such as enhancement or quenching. Analyzing these changes in fluorescence signals facilitates the determination of the presence or degree of degradation of target molecules. Absorbance measurement entails forming complexes between target molecules and dyes within the sensor. DNase II catalyzed degradation of these target molecules modifies the dye's absorbance, detectable through spectral measurement. Similar to previous sections, electrochemical detection involves monitoring changes in electrochemical signals (current or potential) resulting from DNase II catalyzed degradation of the target molecules. The DNase II sensor, akin to its counterparts, leverages the interaction between DNase II and target molecules to swiftly and accurately detect specific molecules or cells. The sensor's high specificity and sensitivity position it for potential applications in biomedical research, clinical diagnosis, and environmental monitoring. (Fig.2)

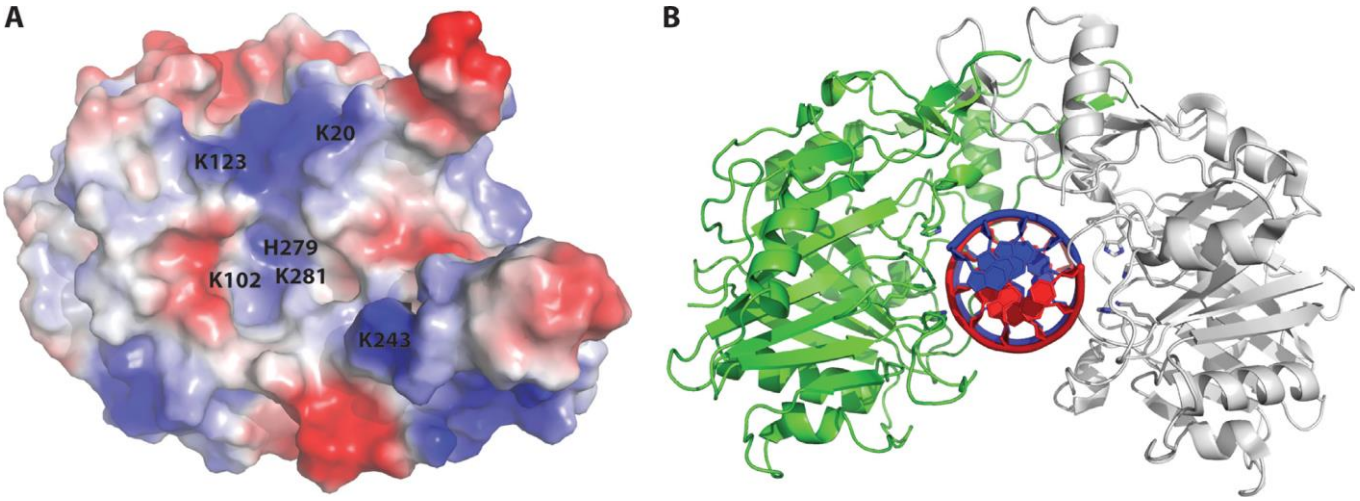

Figure 2. Hypothetical model for DNA recognition by DNase II.

Note: (A) Surface electrostatics of a single DNase II protomer viewed from the interior of the homodimeric U-shaped clamp-like structure. Catalytic residues K102, H279, and H281 are visible from this vantage point, whereas H100 is not. Additional positively charged residues which line the central cavity K20, K123 and K243 are labeled. (B) Hypothetical model for DNA recognition by DNase II generated via manual docking of an ideal B-form DNA into the central cavity of the DNase II homodimer. (Nucleic Acids Res 2017, 45(10):6217-6227.)

4.3. Other DNA Enzyme Sensors

Beyond DNase I and DNase II sensors, many other DNA enzyme-based sensors have been devised for diverse detection purposes. The exonuclease sensor relies on exonucleases-an enzyme class capable of catalyzing the degradation reaction from the ends of DNA strands. Exonuclease sensors are adept at detecting the presence or degree of DNA sequence degradation [25]. Typically, these sensors involve DNA substrates that degrade under exonuclease catalysis. Detection signals can be captured via fluorescence, absorbance, or electrochemical methods. Similarly, the endonuclease sensor capitalizes on endonucleases-enzymes that catalyze the degradation reaction within DNA strands. This sensor detects the presence or degradation of specific DNA sequences. The sensor usually employs a specific DNA substrate. Upon endonuclease binding and catalytic degradation, changes in signal can be detected to indicate the presence of target molecules. (Fig.3)

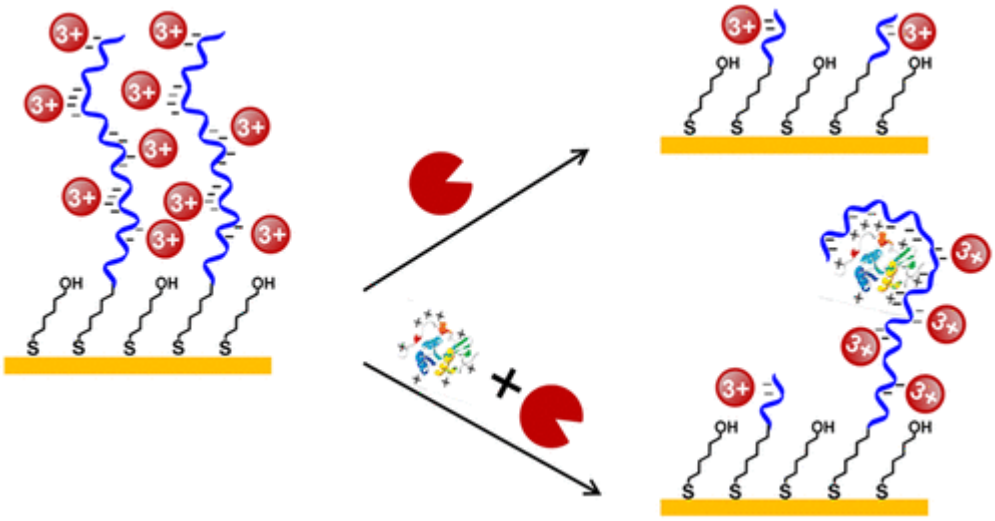

Figure 3. Exonuclease I-Assisted General Strategy to Convert Aptamer-Based Electrochemical Biosensors from "Signal-Off" to "Signal-On".

Note: In terms of how the signal varies in response to increased concentration of an analyte, sensors can be classified as either "signal-on" or "signal-off" format. While both types hold potentials to be sensitive, selective, and reusable, in many situations "signal-on" sensors are preferred for their low background signal and better selectivity. In this study, with the detection of lysozyme using its DNA aptamer as a trial system, for the first time we demonstrated that such an aptamer-based electrochemical biosensor can be converted from intrinsically "signal-off" to "signal-on" with the aid of a DNA exonuclease. The fact that the stepwise cleavage of antilysozyme aptamer catalyzed by Exonuclease I (Exo I) is entirely inhibited upon binding lysozyme leads to the selective removal of unbound DNA probes (thiolate anti-lysozyme DNA aptamer strands immobilized on gold electrode) upon the introduction of Exo I to the sensor. With the aid of electrostatically bound redox cations ([Ru(NH3)6]3+), we were able to quantitate the number of aptamer strands that are bound with lysozymes via conventional cyclic voltammetry (CV) measurements. We demonstrated that Exo I-assisted signal-on conversion protocol not only improves the sensing performance (10 times better limit of detection) but also promises a versatile strategy for DNA-based biosensor design, i.e., it can be readily adapted to other aptamer-protein binding systems (thrombin, as another example). (Anal Chem 2020, 92(9):6229-6234.)

Ligases, enzymes capable of joining two DNA molecules, are utilized in the ligase sensor to detect specific DNA sequences. This sensor often employs two DNA probes. Upon the existence of the target DNA sequence, the ligase connects the two probes. Detection signals, often measured through fluorescence, absorbance, or electrochemical methods, can signify the occurrence of connection events.

The polymerase sensor exploits the synthesizing ability of polymerases-an enzyme class capable of generating new DNA strands. This sensor detects the presence or amplification extent of DNA sequences. Typically, these sensors involve DNA substrates and primers. When the target DNA sequence is present, polymerases synthesize new DNA strands guided by primers. Detection signals, often captured through fluorescence, absorbance, or electrochemical methods, can indicate new DNA strand generation. These DNA enzyme-based sensor approaches exhibit notable specificity and sensitivity, and their applications span a broad spectrum-from biological analysis to medical diagnostics and environmental monitoring. Through sustained research and technological innovation, we anticipate further breakthroughs and advancements in the design and application of DNA enzyme sensors.

5. Future Research Directions for Rapid Detection of Pseudomonas aeruginosa Based on DNA Enzyme Sensor

Presently, DNA enzyme sensors exhibit a certain level of sensitivity and specificity in Pseudomonas aeruginosa detection; however, there remains ample room for enhancement. Future research avenues could involve the

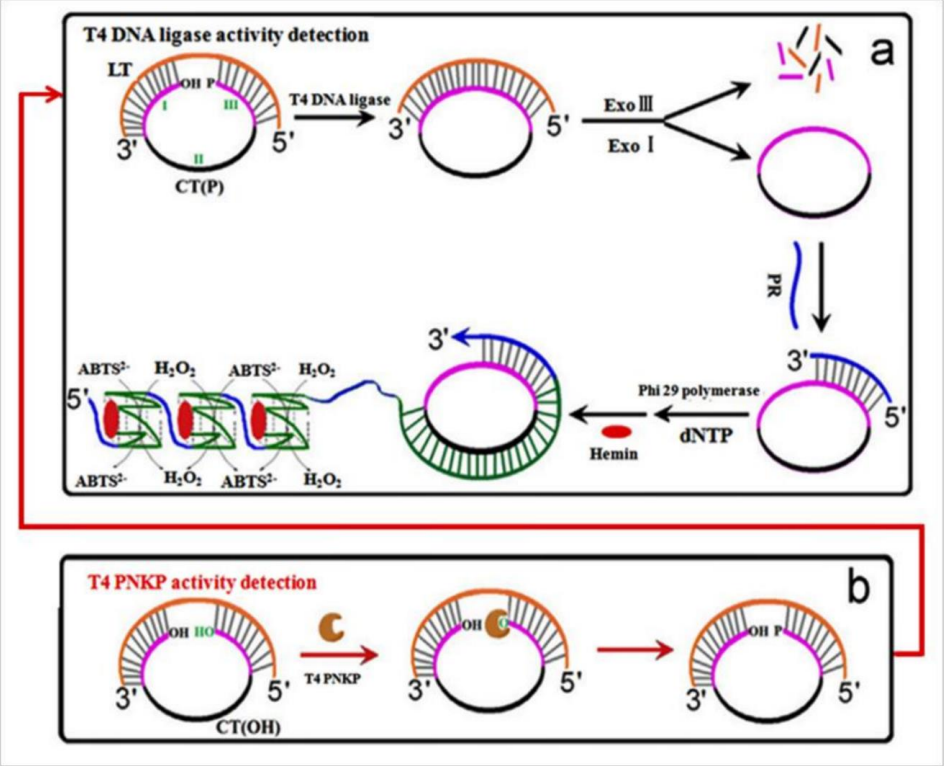

Figure 4. Structure of ligase sensor.

Note: Schematic diagram of G-quadruplex DNAzyme-based DNA ligase sensor. (a) The CT composed of three parts. Part I and III could hybridize with LT and form a split ring. Part II was a Crich area where G-rich sequences are generated to form G-quadruplex structures after RCA. The split was repaired when T4 DNA ligase was introduced; then, LT was excised by Exo I and Exo III After annealing, PR was adhered to the circular template and activated RCA with Phi29 polymerase. Numerous G-rich sequences will be produced to fold into G-quadruplex units and bind hemin to form catalytic G-quadruplex DNAzymes, which can catalyze the oxidation of ABTS 2– by H2O2 to ABTS, enhancing the absorption signal. (b) When the PNKP-triggered 5′-phosphroylation step was added to the substrate DNA, a PNKP sensor was easily designed based on the above sensing strategy. (LT, linear template; CT, circular template; ABTS, 2,2′-azino -bis (3-ethylbenzothiazoline- 6-sulfonic acid)). (Pharmaceuticals (Basel) 2018, 11(2):35.)

exploration of novel DNA enzymes or refining existing enzymes to bolster the sensor's sensitivity and specificity in detecting Pseudomonas aeruginosa. Additionally, optimizing the DNA substrates and primers' design is a crucial research direction to augment the signal amplification effect and the sensor's specific binding capacity.

Future investigations can also delve into pioneering strategies and technologies to achieve swifter and higher-throughput detection methods. Integrating microfluidic technology, nanomaterials, and automated systems could yield efficient and rapid Pseudomonas aeruginosa detection. Developing portable and field-operable detection equipment is paramount for on-site Pseudomonas aeruginosa detection. Thus, future endeavors might be directed towards crafting compact and portable detection apparatuses that integrate sensors and detection systems, facilitating convenient on-site applications across medical, environmental, and food safety domains. The detection of Pseudomonas aeruginosa often necessitates the synthesis of multiple indices and parameters to bolster accuracy and reliability. To this end, integrating DNA enzyme-based sensors with other detection technologies, such as optical, electrochemical, and mass spectrometry, could enable comprehensive multi-parameter analysis. This holistic approach could offer a more comprehensive evaluation of Pseudomonas aeruginosa's existence and activity.

While DNA enzyme-based sensors have succeeded in laboratory settings, their real-world performance and reliability require further validation and assessment. To this end, future research should encompass the application

of these sensors to actual samples, subjecting them to testing and comparison against standard methods to ascertain their feasibility and reliability. By enhancing sensitivity and specificity, developing rapid and high-throughput detection techniques, crafting portable and field-ready equipment, enabling multi-parameter analysis, and corroborating real-world scenarios, the application of DNA enzyme-based sensors in Pseudomonas aeruginosa detection can be further advanced. These research directions are poised to bolster Pseudomonas aeruginosa's rapid detection capabilities, offering more dependable solutions for clinical diagnoses, environmental monitoring, and food safety.

6. Summary and Prospects

The DNA enzyme sensor, built upon the specific binding of DNA enzyme and target molecules, coupled with catalytic degradation reactions, epitomizes a potent method for swift and accurate detection. Within Pseudomonas aeruginosa detection, the DNA enzyme sensor presents notable advantages and promising prospects. The threat posed by Pseudomonas aeruginosa's multi-drug resistance and biofilm formation to human health is substantial. Conventional detection approaches are protracted and intricate. DNA enzyme sensors capitalize on the catalytic degradation reaction's signal changes, facilitated by the DNA enzyme's specific binding affinity with Pseudomonas aeruginosa. Past research has unveiled progress in sensors relying on DNase I, DNase II, and other DNA enzymes for Pseudomonas aeruginosa detection. These sensors boast merits like specificity, sensitivity, real-time monitoring, simplicity, and user-friendliness, finding utility in medical, environmental, and food safety sectors. Nevertheless, DNA enzyme sensors confront challenges-enhancing sensitivity and specificity and refining experimental conditions stand out. Prospective research could emphasize bolstering sensor performance and stability to suit clinical contexts. Ultimately, DNA enzyme sensors harbor extensive prospects for expediting Pseudomonas aeruginosa detection, offering valuable insights into crafting more practical, swift, and reliable detection methodologies.

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Conflict of interest

All the authors claim that the manuscript is completely original. The authors also declare no conflict of interest.

References

1. Islan GA, Ruiz ME, Morales JF, et al. (2017). Hybrid inhalable microparticles for dual controlled release of levofloxacin and DNase: physicochemical characterization and in vivo targeted delivery to the lungs. *J Mater Chem B* 5, 3132-3144. https://doi.org/10.1039/C6TB03366K
2. Belfield K, Bayston R, Hajduk N, et al. (2017). Evaluation of combinations of putative anti-biofilm agents and antibiotics to eradicate biofilms of Staphylococcus aureus and Pseudomonas aeruginosa. *J Antimicrob Chemother* 72, 2531-2538. https://doi.org/10.1093/jac/dkx192
3. Aleanizy FS, Alqahtani FY, Eltayb EK, et al. (2021). Evaluating the effect of antibiotics sub-inhibitory dose on Pseudomonas aeruginosa quorum sensing dependent virulence and its phenotypes. *Saudi J Biol Sci* 28, 550-559. https://doi.org/10.1016/j.sjbs.2020.10.040
4. Nair A, Perry A, Perry JD, et al. (2020). In vitro effects of combined iron chelation, antibiotics and matrix

disruption on clinical isolates of Pseudomonas aeruginosa. *J Antimicrob Chemother* 75, 586-592. https://doi.org/10.1093/jac/dkz505

5. Chen Y, Mei Y, Zhao X, et al. (2020). Reagents-Loaded, Automated Assay that Integrates Recombinase-Aided Amplification and Cas12a Nucleic Acid Detection for a Point-of-Care Test. *Anal Chem* 92, 14846-14852. https://doi.org/10.1021/acs.analchem.0c03883
6. Atack JM, Guo C, Yang L, et al. (2020). DNA sequence repeats identify numerous Type I restriction-modification systems that are potential epigenetic regulators controlling phase-variable regulons, phasevarions. *Faseb j* 34, 1038-1051. https://doi.org/10.1096/fj.201901536RR
7. Kyung Lee M, Armstrong DA, Hazlett HF, et al. (2021). Exposure to extracellular vesicles from Pseudomonas aeruginosa result in loss of DNA methylation at enhancer and DNase hypersensitive site regions in lung macrophages. *Epigenetics* 16, 1187-1200. https://doi.org/10.1080/15592294.2020.1853318
8. Hu Y, He Y, Lin Z. (2020). Biochemical and structural characterization of the Holliday junction resolvase RuvC from Pseudomonas aeruginosa. *Biochem Biophys Res Commun* 525, 265-271. https://doi.org/10.1016/j.bbrc.2020.02.062
9. Fanaei Pirlar R, Emaneini M, Beigverdi R, et al. (2020). Combinatorial effects of antibiotics and enzymes against dual-species Staphylococcus aureus and Pseudomonas aeruginosa biofilms in the wound-like medium. *PLoS One* 15, e0235093. https://doi.org/10.1371/journal.pone.0235093
10. Azzouz L, Cherry A, Riedl M, et al. (2018). Relative antibacterial functions of complement and NETs: NETs trap and complement effectively kills bacteria. *Mol Immunol* 97, 71-81. https://doi.org/10.1016/j.molimm.2018.02.019
11. Hojjati M, Behabahani BA, Falah F. (2020). Aggregation, adherence, anti-adhesion and antagonistic activity properties relating to surface charge of probiotic Lactobacillus brevis gp104 against Staphylococcus aureus. *Microb Pathog* 147, 104420. https://doi.org/10.1016/j.micpath.2020.104420
12. Kumara SS, Bashisht A, Venkateswaran G, et al. (2019). Characterization of Novel Lactobacillus fermentum from Curd Samples of Indigenous Cows from Malnad Region, Karnataka, for their Aflatoxin B(1) Binding and Probiotic Properties. *Probiotics Antimicrob Proteins* 11, 1100-1109. https://doi.org/10.1007/s12602-018-9479-7
13. Jin H, Roy U, Lee G, et al. (2018). Structural mechanism of DNA interstrand cross-link unhooking by the bacterial FAN1 nuclease. *J Biol Chem* 293, 6482-6496. https://doi.org/10.1074/jbc.RA118.002171
14. Rastogi S, Mittal V, Singh A. (2021). Selection of Potential Probiotic Bacteria from Exclusively Breastfed Infant Faeces with Antagonistic Activity Against Multidrug-Resistant ESKAPE Pathogens. *Probiotics Antimicrob Proteins* 13, 739-750. https://doi.org/10.1007/s12602-020-09724-w
15. Ricci A, Coppo E, Barbieri R, et al. (2017). The effect of sub-inhibitory concentrations of rifaximin on urease production and on other virulence factors expressed by Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa and Staphylococcus aureus. *J Chemother* 29, 67-73. https://doi.org/10.1080/1120009X.2016.1195069
16. Bhongir RK, Kasetty G, Papareddy P, et al. (2017). DNA-fragmentation is a source of bactericidal activity against Pseudomonas aeruginosa. *Biochem J* 474, 411-425. https://doi.org/10.1042/BCJ20160706
17. Niazi M, Florio TJ, Yang R, et al. (2020). Biophysical analysis of Pseudomonas-phage PaP3 small terminase suggests a mechanism for sequence-specific DNA-binding by lateral interdigitation. *Nucleic Acids Res* 48, 11721-11736. https://doi.org/10.1093/nar/gkaa866
18. Kim S, Kim SH, Ahn J, et al. (2019). Crystal Structure of the Regulatory Domain of MexT, a Transcriptional Activator of the MexEFOprN Efflux Pump in Pseudomonas aeruginosa. *Mol Cells* 42, 850-857. https://doi.org/10.14348/molcells.2019.0168
19. Patel KK, Surekha DB, Tripathi M, et al. (2019). Antibiofilm Potential of Silver Sulfadiazine-Loaded Nanoparticle Formulations: A Study on the Effect of DNase-I on Microbial Biofilm and Wound Healing Activity. *Mol Pharm* 16, 3916-3925. https://doi.org/10.1021/acs.molpharmaceut.9b00527
20. Reyneke B, Ndlovu T, Khan S, et al. (2017). Comparison of EMA-, PMA- and DNase qPCR for the determination of microbial cell viability. *Appl Microbiol Biotechnol* 101, 7371-7383. https://doi.org/10.1007/s00253-017-8471-6
21. Baraquet C, Harwood CS. (2017). Use of Nonradiochemical DNAse Footprinting to Analyze c-di-GMP Modulation of DNA-Binding Proteins. *Methods Mol Biol* 1657, 303-315. https://doi.org/10.1007/978-1-4939-7240-1_24
22. Liu X, Zhang L, Xiu Y, et al. (2021). Insights into the dual functions of AcrIF14 during the inhibition of type I-F CRISPR-Cas surveillance complex. *Nucleic Acids Res* 49, 10178-10191. https://doi.org/10.1093/nar/gkab738

23. Shahri FN, Izanloo A, Goharrizi M, et al. (2022). Antimicrobial resistance, virulence factors, and genotypes of Pseudomonas aeruginosa clinical isolates from Gorgan, northern Iran. *Int Microbiol* 25, 709-721. https://doi.org/10.1007/s10123-022-00256-7
24. Johnston EL, Zavan L, Bitto NJ, et al. (2023). Planktonic and Biofilm-Derived Pseudomonas aeruginosa Outer Membrane Vesicles Facilitate Horizontal Gene Transfer of Plasmid DNA. *Microbiol Spectr* 11, e0517922. https://doi.org/10.1128/spectrum.05179-22
25. Costa KC, Glasser NR, Conway SJ, et al. (2017). Pyocyanin degradation by a tautomerizing demethylase inhibits Pseudomonas aeruginosa biofilms. *Science* 355, 170-173. https://doi.org/10.1126/science.aag3180