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Review

Bioanalytical applications of isothermal nucleic acid amplification techniques

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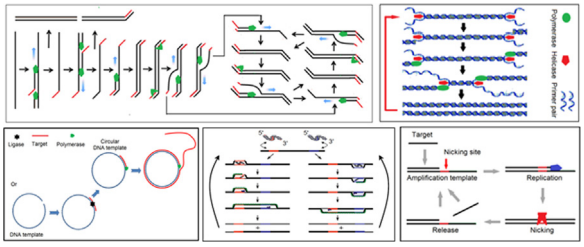
HIGHLIGHTS

- Eminent properties of eleven isothermal nucleic acid amplification techniques are highlighted.
- The applications of the isothermal nucleic acid amplification techniques in bioanalytical chemistry are extensively reviewed.
- Comparison between PCR and the isothermal nucleic acid amplification techniques is attempted.
- Comparison amongst the isothermal nucleic acid amplifications is attempted.
- Future perspectives of the isothermal nucleic acid amplification techniques are discussed.

GRAPHICAL ABSTRACT



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ABSTRACT

The most popular in vitro nucleic acid amplification techniques like polymerase chain reaction (PCR) including real-time PCR are costly and require thermocycling, rendering them unsuitable for uses at point-of-care. Highly efficient in vitro nucleic acid amplification techniques using simple, portable and low-cost instruments are crucial in disease diagnosis, mutation detection and biodefense. Toward this goal, isothermal amplification techniques that represent a group of attractive in vitro nucleic acid amplification techniques for bioanalysis have been developed. Unlike PCR where polymerases are easily deactivated by thermally labile constituents in a sample, some of the isothermal nucleic acid amplification techniques, such as helicase-dependent amplification and nucleic acid sequence-based amplification, enable the detection of bioanalytes with much simplified protocols and with minimal sample preparations since the entire amplification processes are performed isothermally. This review focuses on the isothermal nucleic acid amplification techniques and their applications in bioanalytical chemistry. Starting off from their amplification mechanisms and significant properties, the adoption of isothermal amplification techniques in bioanalytical chemistry and their future perspectives are discussed. Representative examples illustrating the performance and advantages of each isothermal amplification technique are discussed along with some discussion on the advantages and disadvantages of each technique.

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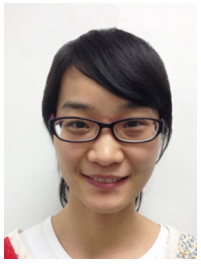

Ms. Huimin Deng was born in China. In 2011, she received her B.Sc. in Chemistry from Sichuan University. She is currently pursuing her Ph.D. study at the National University of Singapore. Her research is primarily on the development of electrochemical biosensors and fluorescence bioassays.

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1. Introduction

Enzyme-catalyzed deoxyribonucleic acid (DNA) replication is an indispensable process in all living organisms as it replicates copies of genetic instruction that is essential to sustain life. More importantly, the replication strategies have long been optimized over the course of evolution. As a consequence, this enzyme-based DNA replication process, and polymerase chain reaction (PCR) in particular, has been widely exploited as an in vitro nucleic acid amplification strategy in molecular biology and bioanalysis. PCR engages the reiterative cycling of a reaction cocktail between different temperatures to achieve amplification [1]. In principle, the reiterative cycling of heating and cooling is able to achieve a two-fold amplification in each cycle.

Being one of the most popular nucleic acid amplification techniques, PCR is most frequently used for the amplification of traces of nucleic acids. PCR is known for its long turnaround time in the past, the advent of technology and automation has significantly reduced the PCR reaction time [2–4]. It is also possible to achieve relatively high throughput by using microplates and multiplex PCR processes in each well. In addition, whole genome amplification is now possible by using random primers [5–7]. However, the PCR technique suffers from several drawbacks such as easy contamination, high cost, susceptibility to false amplification and tendency for sequence mismatches with typical error rates of 10^{-6} depending on the polymerase [8]. Furthermore, it also requires highly purified nucleic acid samples in order to sustain the activity of the polymerase [9]. These disadvantages, coupled with the compulsory requirement of a thermal cycler, which causes serious problems in the miniaturization of PCR instruments, have encouraged researchers to develop isothermal nucleic acid amplification techniques that can circumvent some of these drawbacks, yet possess the strength of PCR. Therefore, non-PCR-based nucleic acid amplification techniques and instruments have attracted tremendous attention and impressive progress has been made over the years. It has been shown that non-PCR-based clinical tests often outperform PCR tests in clinical diagnosis [10,11]. Among them, isothermal nucleic acid amplification techniques offer the solution to the drawbacks of their PCR-based amplification counterparts.

In the isothermal nucleic acid amplification techniques, for example helicase-dependent amplification (HDA), nucleic acid amplification is carried out at a constant temperature although an initial temperature ramping may be necessary in some cases. Isothermal nucleic acid amplification techniques completely remove the requirement of a thermal cycler, thus significantly simplifying the protocols for uses at point-of-care. It also infers that the techniques are easier to use, more cost effective and more tolerant of inhibitory components from pristine samples than PCR [11–15]. An additional advantage of isothermal nucleic acid amplification is that some of them allow the use of non-DNA targets such as messenger RNA (mRNA) or even thermally labile protein-nucleic acid conjugates [16–18]. For instance, a number of ultrasensitive protein assays have been developed by coupling immune-detection with isothermal nucleic acid amplification [19–21]. Moreover, whole genome amplification and analysis have been achieved and large quantities (1–10 μg) of amplified products can be produced from single cells. On the other hand, major challenges in isothermal nucleic acid amplification techniques include the lack of diversified detection techniques and low multiplexing capabilities.

Isothermal nucleic acid amplification techniques can be categorized into two groups – target amplification and target recycling amplification – and the majority of the isothermal amplification techniques belong to the former. In addition to reviewing the general aspects of some of the isothermal nucleic