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REVIEW

Application Progress of Exonuclease-Assisted Signal Amplification Strategies in Biochemical Analysis

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Abstract: As an important member of the tool enzymes, exonuclease is a kind of hydrolytic enzymes without strict base sequence dependence. In recent years, by taking advantage of different hydrolysis ways of exonuclease and nanotechnology, cycle effect of enzyme digestion, aptamer, non Watson-Crick base pairing system by metal ions, fluorescent nucleic acid probes, electrochemical methods etc., a series of exonuclease-assisted signal amplification strategies have been developed, which have played a key role in improving the sensitivity of detection method. Therefore, exonucleases have been widely used in highly sensitive detection of nucleic acids, proteins, ions, small molecules and so on. To understand it better and apply it well in the future, the application progresses of exonuclease-assisted signal amplification strategies in biochemical analysis have been summarized in this review.

Key Words: Analysis and detection; Tool enzymes; Signal amplification; Exonuclease; Review

1 Introduction

Rapid, accurate and sensitive detection of ions, molecules, nucleic acids and proteins is of great importance in diseases prevention and diagnosis, environmental monitoring, food safety monitoring and so on. In the actual biochemical analysis, the presence of targets analytes is usually in trace amounts, thus the analytical methods with high sensitivity and high accuracy are required. For this purpose, in addition to the development of direct and highly sensitive methods, the amplification detection strategy can reduce the degree of difficulty in the design of the analytical methods or sensors as well as the dependence on the large, specialized instruments. According to the objectives of amplifications, the detection methods can be divided into three categories, including target amplification strategies, probe amplification strategies and signal amplification strategies[1]. The target amplification strategies are usually achieved by amplification of targets to the level that can be detected with conventional methods. For example, by using polymerase chain reaction (PCR) as targets amplification strategy, the targets can be amplified by 10^8–10^9 times[2]. For the probe amplification strategies, such as rolling circle amplification (RCA), the amount of targets is constant in the assay system. However, the signal probes are efficiently amplified (amplification efficiency of RCA is 10^3–10^4 times)[3].

Compared to target amplification strategies and probe amplification strategies, signal amplification strategies are more optional. In recent years, many biological and chemical signal amplification strategies have been developed by using different ways, including hybrid chain reaction (HCR), tool enzymes, enzymatic catalytic reaction, nanomaterials, and so on. Among these strategies, the tool enzymes (e.g., polymerase, ligase, endonuclease, exonuclease, ribonuclease) assisted signal amplification strategies have undergone rapid development in biochemical analysis due to the tool enzymes' high sensitivity and specificity, simple operation, and mild reaction conditions[4–9]. In particular, by combining nuclease with nanotechnology, cycle effect of enzyme digestion, aptamer, non Watson-Crick base pairing system by metal ions, fluorescent nucleic acid probes, and electrochemical methods, a series of nuclease-assisted signal amplification strategies

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have been widely used in the detection of nucleic acids, proteins, ions and small molecules[10–14]. There are two major types of nuclease-assisted signal amplification strategies, sequence-dependent restriction enzymes-assisted amplification strategies and sequence-independent exonuclease-assisted amplification strategies. From the standpoint of universality, sequence-independent exonuclease shows a wider application prospect due to its better execution of probes design and targets analysis[15]. This review firstly introduces the classification and characteristics of exonuclease, and then summarizes the application progress of the exonuclease-assisted signal amplification strategies in detection of nucleic acids, proteins, small molecules and ions. Finally, the challenges and developments in the future have also been discussed briefly.

2 Classification and characteristics of exonucleases

Exonucleases are a kind of tool enzymes which can cleave nucleotides one at a time from the end (3' or 5') of a polynucleotide chain and produce nucleotides (DNA-dNMPs, RNA-NMPs). According to the specificity of the substrates, exonucleases could be divided into two types, single-stranded DNA (ssDNA) specific exonuclease (e.g., exonuclease I and exonuclease VII) and double-stranded DNA (dsDNA) specific exonuclease (e.g., exonuclease III, λ exonuclease, T7 exonuclease). Although exonucleases are one kind of sequence-independent enzymes, different exonucleases have their specific hydrolysis characteristics. In order to get a better understanding of the exonuclease-assisted signal amplification strategies, we here list several main exonucleases that have been commonly used in bioanalyses. The hydrolysis characteristics of these exonucleases are shown in Table 1 and Fig.1. Taking exonuclease III as an example, the preferred substrates are dsDNA with blunt or recessed 3′-termini, and exonuclease III

Table 1 Several main exonucleases and their hydrolysis characteristics

Exonuclease	Substrates	Hydrolysis direction and site requirements	Product
Exonuclease I (*E. coli*)	ssDNA	3′→5′ (3′-OH)	5′-nucleotide
Exonuclease Ⅶ (*E. coli*)	ssDNA	3′→5′ (3′-OH) 5′→3′ (5′-P)	2-12 bp oligonucleotide fragment
Exonuclease III (*E. coli*)	dsDNA	3′→5′ (3′-OH blunt or recessed)	5′-nucleotide + single stranded oligonucleotide
λ Exonuclease	dsDNA	5′→3′ (5′-P)	5′-nucleotide + single stranded oligonucleotide
T7 Exonuclease	dsDNA	5′→3′ (5′-OHor 5′-P)	5′-nucleotide + single stranded oligonucleotide

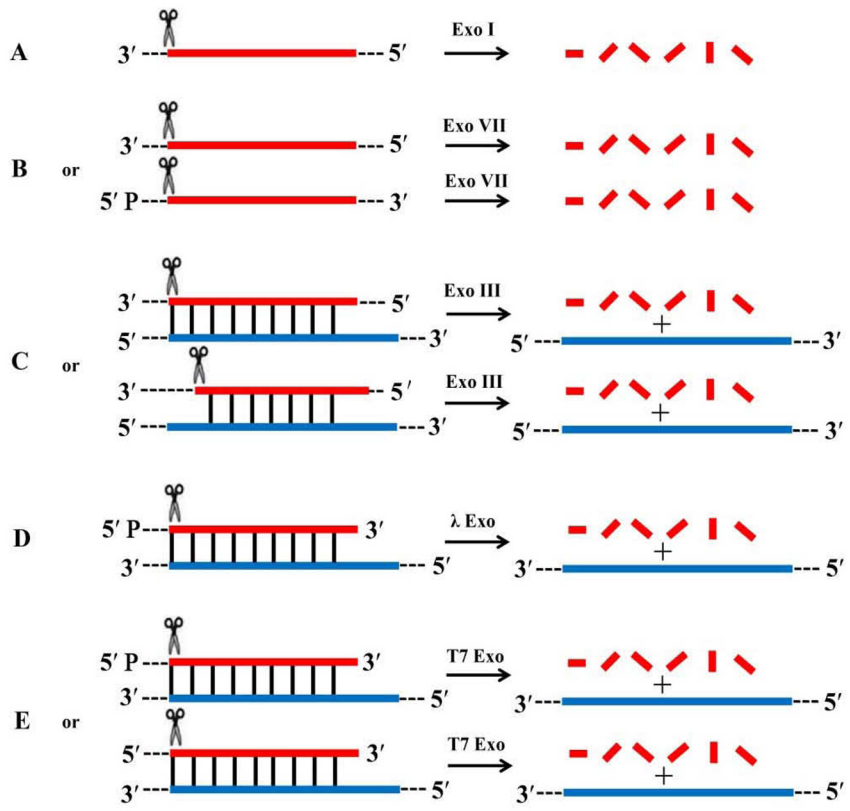

Fig.1 Basic characteristics diagram of several exonucleases

A, exonuclease I (Exo I); B. exonuclease VII (Exo VII); C. exonuclease III (Exo III); D. λ exonuclease (λ Exo); E. T7 exonuclease (T7 Exo)