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Perspective

Comprehensive Study on Nucleic Acid Detection in Enzyme-Labeled Probes

Jonathan Feng*

Department of Chemistry, Lomonosov Moscow State University, Moscow, Russia

INTRODUCTION

Nucleic acids can be labelled with detection or purification tags. The generated nucleic acid probes can be used to locate and recover other interacting molecules. Labels for nucleic acid probes include radioactive phosphates, biotin, fluorophores, and enzymes. Using the same bio conjugation techniques used to create nucleic acid probes, nucleic acids can also be attached to other molecules or surfaces to enable targeted distribution or immobilization. There are several reagents available for quick and effective benchtop oligonucleotide labelling, and they work well for producing specific amounts of probe or when multiple probes bearing the same label are needed for mutational analysis. Enzymatic techniques are a low-cost way to label probes for small-scale probe generation.

Larger-scale reactions are possible thanks to chemical methods. Making probes that are tagged at the 5′ or 3′ ends of the oligonucleotide or that are dispersed throughout the sequence can be done chemically or enzymatically. The level of labelling required and whether or not the modification will cause steric hindrance, which prevents the intended interactions, affect the strategy to be used. For nucleic acid hybridization experiments, the high specific activity obtained from the random insertion of label into a probe is typically advantageous. However, in experiments that require protein interactions, like gel shift and pull-down assays, end-labeling is essential to enable protein binding.

Molecular biology research in areas like genetics, genomics, diagnostics, and biotechnology all heavily rely on the identification and analysis of nucleic acids. For studying gene expression, finding genetic mutations, diagnosing illnesses, and comprehending the mechanisms of biological processes, accurate and sensitive detection methods are crucial.

DESCRIPTION

Due to their high sensitivity, specificity, and adaptability, enzyme-labeled probes have become effective tools for detecting nucleic acids. These probes have complementary target nucleic acid sequences that they are intended to hybridise with, and they are attached to enzymes that catalyse detectable reactions, allowing the visualization or quantification of the target molecule. This method has completely changed the field of molecular diagnostics and made significant progress in our understanding of genetic diseases and the underlying causes of those diseases.

Several crucial steps are involved in the process of enzyme labeled probe based nucleic acid detection. The first step is isolating the target nucleic acid from the sample of interest. This nucleic acid could be DNA or RNA. This might be blood, tissue, or a sample that has been prepared in a lab. After that, a specific enzyme-labeled probe made to hybridise with the target sequence is added to the isolated target nucleic acid.

Various chemical or enzymatic techniques can be used to conjugate the enzyme to the probe. The preferred detection technique and the available substrates influence the enzyme selection. Horseradish Peroxidase (HRP), Alkaline Phosphatase (AP), and Glucose Oxidase (GOx) are examples of frequently used enzymes.

A hybridization reaction takes place when the enzyme-labeled probe and the target nucleic acid are mixed together, producing a stable probe target complex. Then, any non-specific binding is eliminated by washing away unbound probes. The remaining probe-target complex is after that put through a suitable detection process.

There are numerous methods that can be used to detect enzyme labeled probes. Utilizing chromogenic substrates is one typical strategy. For instance, a chromogenic substrate like 3,3'-Diaminobenzidine (DAB) or 3,3',5,5'-Tetramethylbenzidine (TMB) is added if Horseradish Peroxidase (HRP) is used as the enzyme label. At the site of the probe target hybridization, the substrate undergoes a colorimetric reaction in the presence of the enzyme, resulting in the formation of a colored precipitate. This enables spectrophotometric visual detection or quantification.

Correspondence to: Jonathan Feng, Department of Chemistry, Lomonosov Moscow State University, Moscow, Russia; E-mail: Jonathanfeng@gmail.com

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CONCLUSION

Chemiluminescence based detection is another method. In this technique, a chemiluminescent reaction is catalysed by the enzyme labeled probe, resulting in the emission of light. A specialised device, such as a luminometer or a CCD camera, is then used to detect the light that has been released. This detection method is appropriate for applications requiring increased detection limits because it has a high sensitivity and little background noise.