

A Novel Enzyme for Use in Labeling Biomolecules

Background

Peroxidases and microbial laccases are used in industrial applications such as stain bleaching and anti dye-transfer, in detergents, polymerization of lignin, bleaching of denim and other dyed fabrics, and hair coloring products. In molecular biology and immunohistochemical analysis alkaline phosphatase and peroxidase enzymes are routinely used to detect and estimate DNA, RNA and proteins via the measurement of chemifluorescence or chemiluminescence of end products. However, peroxidase applications require the use of hydrogen peroxide as a cofactor and alkaline phosphatase yields high background.

Another enzyme, catalase, has also been used in some of the industrial and research applications described above. This enzyme has peroxidase (oxidization of alcohols in the presence of hydrogen peroxide) and catalatic (breakdown of hydrogen peroxide to oxygen and water) activities. The peroxidase activity requires the addition of horseradish peroxidase and hydrogen peroxide and the end point used to detect catalase activity entails the monitoring of a decrease in the fluorescence, which compromises the sensitivity of the assay.

The present technology represents a significant improvement to the currently used nucleic acid and protein assays and detection systems. Novel fluorescent probes and methods for their use in research applications are described.

Description of the Technology

It was discovered that the mammalian enzyme catalase has a unique oxidase activity in the presence of certain substrates. This enables the exploitation of this enzyme to oxidize a number of non-fluorescent substrates to produce intensely fluorescent end products without the requirement for hydrogen peroxide as co-factor. Also, the oxidase activity of the enzyme could be competitively inhibited by certain substrates of the enzyme. The optimum conditions and kinetics of the reaction have been deciphered for AMPLEX-RED and DCFH-DA substrate. It was also discovered that the fluorescent signal could be amplified under certain experimental conditions. Methods and techniques for enhancing the fluorescent signal have been worked out.

Advantages

- . • Does not require the addition of peroxidase enzyme and hydrogen peroxide as co-factor
- . • Simple and sensitive assay that can be used to detect very low levels of DNA, RNA and proteins.
- . • Non-radioactive assay
- . • Nucleic acid and protein assays have low background
- . • The fluorescent signal can be amplified enabling the use of this technology in assays requiring sustained signal amplification over long periods of time
- . • This technology can be adapted for colorimetric, chemiluminescent , or chemifluorescent assays for diagnostic and other medical applications

Applications

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- . • Nucleic acid analyses using techniques such as gene profiling, sequencing, southern and northern blots -Kits for the use of the catalase enzyme in each of these applications can be developed using the methods described in the patent application.
- . • Protein analyses using techniques such as western blotting, ELISA, immunohistochemistry with significantly reduced background. Current methods produce significant background, which interferes with the accuracy and consistency of the data obtained by these methods.
- . • In Industrial applications: -preparation of hair coloring products (without hydrogen peroxide) -Detergents-for stain removal -Paper industry-for removal of excess dyes and inks and for bleaching printed paper
- . • Currently, enzymes such as lipase, protease, xylanase are used to deink paper. Catalase may be added to bleach the dyes -Textile industry-for removal of excess dyes without the need for addition of hydrogen peroxide
- . • To produce gelled products in agriculture, pharmaceutical and personal care industry.
- . • In wood industry to produce fiber boards.
- . • As an antimicrobial-antibacterial or antiviral agent

Patent Status

- . • United States patent application filed.
- . • Application published on 12/11/2003 (Publication No.: US-2003-0228648-A1)

Licensing Opportunity

- This technology is available for non-exclusive license.

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