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Application of Lactase Enzymes as Bio-receptors for the Organic Dye Methylene Blue in a Surface Plasmon Resonance Biosensor

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Description

The application of lactase enzymes as bioreceptors in surface plasmon resonance (SPR) biosensors for detecting organic dyes, specifically methylene blue (MB), represents a novel approach to environmental monitoring and biosensor technology. Methylene blue, a commonly used dye in textile and pharmaceutical industries, poses significant environmental concerns due to its toxicity and persistence in aquatic ecosystems. Conventional methods for detecting methylene blue, such as spectrophotometry or chromatography, often require sophisticated equipment, extensive sample preparation, and high costs. In contrast, SPR biosensors offer a label-free, real-time, and highly sensitive detection platform, making them ideal for monitoring organic pollutants like methylene blue [1]. Lactase enzymes, also known as βgalactosidases, are traditionally used in food and pharmaceutical industries for their ability to hydrolyze lactose into glucose and galactose. However, their potential as bioreceptors in biosensors is gaining attention due to their specificity and ability to interact with certain organic compounds, including dyes. The enzyme's structure, featuring an active site that can bind selectively to specific molecules, makes it an excellent candidate for detecting methylene blue. By immobilizing lactase onto the sensor surface, the SPR biosensor can harness its specificity to bind methylene blue, leading to measurable changes in the refractive index near the sensor surface.

The SPR biosensor operates on the principle of surface plasmon resonance, a phenomenon that occurs when polarized light interacts with free electrons in a metal film, typically gold, deposited on the sensor chip. The interaction generates surface plasmons, resulting in a resonance condition that depends on the refractive index of the medium near the metal surface. When methylene blue binds to the immobilized lactase on the sensor surface, it causes a localized change in the refractive index, which can be detected as a shift in the SPR angle or wavelength. This shift is proportional to the concentration of methylene blue, enabling quantitative detection. The construction of the SPR biosensor begins with the preparation of the sensor surface. A thin gold layer is deposited onto a glass substrate to create the SPR-active surface. The gold layer is then functionalized with a self-assembled monolayer of thiols, typically containing carboxyl or amino groups, to provide a suitable platform for lactase immobilization [2]. Lactase is covalently attached to the SAM using crosslinking agents such as carbodiimides, ensuring stable and robust binding. The immobilization process is optimized to preserve the enzyme's activity and orientation, ensuring that its active site remains accessible for binding methylene blue.

The performance of the lactase-based SPR biosensor was evaluated using solutions of methylene blue at various concentrations. The sensor was exposed to these solutions, and the resulting shifts in the SPR signal were recorded in real time. The biosensor demonstrated high sensitivity, with a detection limit in the nanomolar range, which is sufficient for monitoring methylene blue in environmental samples. The response was rapid, with binding events occurring within minutes, and the signal exhibited a linear relationship with methylene blue concentration over a wide dynamic range. Selectivity is a critical parameter for any biosensor, and the lactase-based SPR biosensor showed excellent selectivity for methylene blue over other dyes and organic compounds. This selectivity is attributed to the specific interactions between methylene blue and the active site of lactase, which involve electrostatic interactions and hydrogen bonding. Control experiments using non-specific proteins or unfunctionalized sensor surfaces confirmed that the observed SPR signals were due to specific binding of methylene blue to the lactase bioreceptor.

References

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