Establishing an enzyme assay system based on a surface-enhanced fluorescence method

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【Introduction】

In recent years, the application of metal nanostructures has been investigated in various fields of clinical testing, and their use in surface-enhanced fluorescence (SEF) is also promising. Although there are many reports on measurements of enzymatic reaction products by fluorescence, there are only a few reports on enhanced fluorescence detection by SEF and real-time measurement. We have prepared SEF substrates by adsorbing silica particles on glass slides and coating them with noble metal. After addition of the substrate, the fluorescence intensity of the enzyme reaction product was measured by spatially scanning the excitation light. In this study, PDMS was used to enable real-time measurement of the enzyme reaction. To further stabilize the measurement, we used a thiol linker to immobilize the enzyme onto the surface. Using this technique, a pre-determined amount of the enzyme was adsorbed on the substrate, and the enzyme reaction was monitored in real time.

【 Method 】

Manufacturing method of SEF substrates: Glass slides are immersed in 1 vol% 3-aminopropyl trimethoxy silane (APTMS) solution for 5 min. After drying, the glass slides are immersed in a suspension containing silica particles (100 nm in diameter), and a monolayer of silica particles is formed on the surface of the glass slides. Finally, 100 nm of silver is deposited on the glass slide as the standard procedure. For real-time measurement, PDMS was soaked in alkaline phosphatase (ALP) 3.0×10^{-3} U/mL for 30 min, 1 day, and 3 days. After that, PDMS was washed with PB Buffer (pH 7.4), and NBT/BCIP was added onto the PDMS and then pressed onto the SEF substrate under the weight of 1000 g. The SEF substrate on which the measurement substance was added was fixed on a rotating stage (Shibasaki Seisakusho Co., Ltd.) and rotated at 10 rpm. This prevented bleaching of fluorescence. The SEF substrate was measured with a fiber type fluorescence detector (Nippon Sheet Glass Co., Ltd. Excitation wavelength: 630 nm) for 20 seconds. Next, the substrate surface was immersed in thiol linker (3.0 mM) solution for 60 min. ALP (3.0×10^{-3}, 3.0×10^{-4}, 3.0×10^{-5}, 3.0×10^{-6}, 3.0×10^{-7}U/mL) was added the SEF substrate, washed with water and air-dried. 7.5 μL of NBT/BCIP was added to the SEF substrate and allowed to adsorb for 15 min. The SEF substrate was fixed onto the stage and the signal was measured for 20 seconds.

【Result 】

The enzymatic reaction measurement using PDMS showed that the peak of the enzymatic reaction was observed after 25, 20, and 10 min, corresponding to soaking time of 30 min, 1 day, and 3 days. Furthermore, the longer the soaking time, the more stable the fluorescence intensity was. ALP (3.0×10^{-3}, 3.0×10^{-4}, 3.0×10^{-5}, 3.0×10^{-6}, 3.0×10^{-7} U/mL) and NBT/BCIP were reacted. All the fluorescence intensities were about 15 a.u. when measured at an excitation wavelength of 630 nm. Comparison of the thiol linker-adsorbed substrate with the normal SEF substrate showed a nearly 100-fold difference in the limit of detection. While the optical fiber of the fluorescence detector used in this experiment did not allow independent control of the excitation light and fluorescence intensity, we plan to solve the problem of fading due to excitation light by selecting an optimized cutoff filter.

References:

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