A Novel Method for Fabrication of a Glass-Electrode-Based Lipase Sensor

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Abstract: Based on an unusual reversible sol-gel transition phenomenon, a novel method for the fabrication of a lipase electrode was developed. The response characteristics of the biosensor was studied by potentiometric technique using olive oil as substrate. After optimization, the lipase electrode demonstrated high activity and good stability.

Keywords: Lipase, microemulsion-based gel, biosensor, response characteristics.

Lipase is one of the hydrolases. It catalyzes the hydrolysis of triglycerides, liberating fat acids. Based on its catalysis and the response of a glass electrode to the acids, a glass-electrode-based lipase sensor can be fabricated[1], which has great potential in clinical medicine for the detection of blood fat in human serum samples.

The immobilization of lipase on the bulb of a glass electrode is the key to the development of the sensor. In addition to its response time, which has a lot to do with the thickness of the active membrane, the performance of the sensor depends, to a great extent, on the activity and stability of the immobilized lipase, which are closely related to its microenvironment. Lipase is a surface activated enzyme and, therefore, interface plays an important role in the course of catalysis. Its natural environment can hardly be mimicked by traditional methods of enzyme immobilization with the exception of microemulsion-based gels[2,3].

Microemulsion (microemulsion at low water content is also referred to as reverse micelles) is an organic microheterogeneous medium composed of surfactant, water and non-polar organic solvent. As a result of its peculiar microdomain, enzymes hosted in the water pool of reverse micelles acquire novel physical and conformational properties. During the past 20 years, enzyme catalysis in reverse micelles, *i.e.,* micellar enzymology, has been a subject of great interest[4,5]. For most enzymes, they show high activity and good stability in such medium. These results, together with the findings made in the late 1980s that water-in-oil microemulsion could be gelatinized[2], open up possibilities for the development of novel biosensor. The novel materials obtained based on the gelation of gelatin in water-in-oil microemulsion is known as organogel, hydrocarbon gel, or microemulsion-based gel (MBG). An immobilized lipase on MBG has been prepared

and used as biocatalyst in biosynthesis and biotransformation in organic solvents[6-9]. But there is no report on the application of the normal sol-gel phenomenon for the development of biosensor. One reason may be that the normal sol formed at high temperature(>35°C) is too viscous to obtain even a thin active coating on the bulb of the glass electrode. A novel phenomenon[10], observed by Fadnavis *et al.* in 1999 during their studies on the solubilization of gelatin in microemulsion, attracts our attention. They found that organogels at high surfactant concentrations had an unusual reversible sol-gel transition phenomenon; *i.e.,* the highly viscous organogels obtained at temperature above 30°C became free-flowing liquid at low temperature (5~10°C). Based on this reversible temperature-dependent sol-gel transition phenomenon, a lipase sensor has been fabricated in our lab. After optimization, the biosensor demonstrates good performance. Fabrication procedure of the lipase sensor and its response characteristics are described in this letter.

Fabrication of lipase electrode

Procedures for the construction of a glass-electrode-based lipase sensor are as follows:

1) A water-in-oil (w/o) microemulsion of bis-(2-ethylhexyl) sulfosuccinate sodium (AOT) (Sigma), triply-distilled water, isooctane was prepared by mixing 8.55 mL of 0.30 mol/L AOT in isooctane with 1.45 mL of water (the mixture became clear quickly).

2) A microemulsion-based sol was prepared, first, by adding 0.60 g of gelatin(G-2500, Sigma) to the w/o microemulsion(10.0 mL), then, by heating the above mixture in a water bath at *ca.* 50°C under vigorous stirring until it became cloudy and viscous (approximately 30 min), and finally, by cooling the hot mixture in ice-water bath with stirring for 10 min to get a transparent free-flowing liquid.

3) Lipase powder(L-1754, Sigma) (20 mg) was added to the cold free-flowing liquid with stirring and dissolved into the liquid.

4) A dry but pretreated glass electrode was dipped into the lipase-containing cold sol for 2~3s, then taken out and gently rotated around its axis for 2~3 min at room temperature (*ca.* 25°C). This immobilization step was repeated for three times at intervals of 15 min.

5) The active coating on the bulb was retained. To prevent swelling of the coating and leakage of the enzyme, the coated bulb was re-coated with a thin layer of cellulose acetate by dipping it into a solution of 2% (w/v) cellulose acetate in acetone for 2~3 s at room temperature. After that, the coated electrode was dried under vacuum for 12 h.

6) The biosensor was immersed in the buffer of 0.025 mol/L (pH = 8.5) and stored in a refrigerator (*ca.* 4°C). It was then ready for use.

Recommended procedure for potential measurement

Potential measurements were made at 37±0.1°C on a pHS-3C pH/mV-meter using the lipase electrode as indicator (connected to the negative pole), a calomel electrode as reference (connected to the positive pole), and a Tris-HCl buffer (pH = 8.5) as medium. After the attainment of a steady-state potential response to the blank(29.0 mL), 1.0 mL of

2.58×10^{-3} mol/L olive oil (Sigma) emulsion (its preparation method was reported elsewhere[1]) was added quickly. Ten minutes later, a potential was recorded. Between measurements, the lipase electrode was rinsed with distilled water and kept in the buffer.

Performance of the biosensor

Effects of such variables as were used for fabricating protection membrane on the response time were studied. Also studied were effects of such parameters as the composition of w/o microemulsion, the weight percentage of gelatin in the matrix, the amount of lipase, and the pH value and the temperature of the medium for measurements on the potential difference with and without olive oil ($\triangle E$) (data not shown here). After optimization, a steady-state potential was attained in 5~10 min. A plot of $\triangle E$, measured in the pH 8.5 Tris-HCl buffer at 37°C, *versus* the logarithm of the concentration of olive oil (C_{olive}) was linear over the range from 2.2×10^{-5} mol/L to 2.0×10^{-3} mol/L with the slope being 41.8 mV/pC_{olive}. For an 8.6×10^{-5} mol/L olive oil emulsion (final concentration), the relative standard deviation (RSD) of seven replicate determinations was 2.8%. The stability of the sensor was also studied by monitoring its response, every other day, to a given olive oil (8.6×10^{-5} mol/L) emulsion. No significant change in response characteristics was observed after 15 replicate determinations made every other day. The lipase sensor is relatively stable for 30 days. Forty-five days later, however, only 60% of its initial response remained.

Application potential

Accurate and rapid determination of serum triglycerides is of great significance in clinical medicine. All existing methods are based on the determination of glycerol liberated from triglycerides after hydrolysis. As compared with the chemical method, the enzymatic method is time-saving. However, it is subject to the interference of endogenous free glycerol and significant error occurs for samples from those people who are diabetics and/or suffer from liver diseases. This problem can be avoided if the lipase electrode presented here was used. Attempts were made to apply this electrode to the detection of triglycerides in human serum samples.

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