Preparation of Fine Implantable Needle-Type Glucose Lactate Dual Biosensors Using γ-Polyglutamic Acid

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γ-Polyglutamic acid (PGA) is a non-animal origin biodegradable polyamino acid, which is known as the sticky paste formed on the surface of fermented soybeans "Natto". Since PGA is a low cost, non-toxic, water-retentive material, and also edible toward humans and environment, it has been applied as a material in various fields, such as cosmetics, food, plastics and flocculant for water treatment. Recently, PGA has attracted a great deal of attention for its good biocompatibility, and the researches of PGA for clinical applications, such as bioglue, drug delivery system (DDS) and tissue engineering are prosperous. Considering that PGA is a polyamide with numerous carboxyl groups on the side chain, it seems to be suitable for a material of enzyme immobilization, since carboxyl group can easily form a covalent binding with lysine residue of the enzyme in the presence of a condensation agent. Recently, we have reported that PGA can be applied to immobilize glucose oxidase (GOx) and the obtained GOx-immobilized electrode will function to measure glucose in horse serum [1]. On the other hand, lactate is a compound produced in a process of fermentation during normal metabolism and exercise. It is also a useful index to determine the status of the acid base homeostasis in the body. Therefore, development of glucose/lactate dual biosensor is interesting for clinical application. In this study, PGA was applied for not only the immobilization of GOx, but also lactate oxidase (LOx) and glucose/lactate dual-channel needle type biosensor was fabricated in a single fine needle with individually separated two sensing regions.

The schematic illustration of dual biosensor is shown in Fig. 1. The Pt-Ir (φ 0.1 mm) wire and Pt-coated polyimide tube (φ 0.22 mm) with a Pt thickness of 20 nm were used as 1th (lactate) and 2th (glucose) sensing regions, respectively. In order to inhibit the influence of the electrochemical activity species exists in biological fluid, γ-PGA and cellulose acetate were used as an inner layer. Immobilization of enzymes (glucose oxidase and lactate oxidase) were performed by the condensing reaction between carboxyl groups in γ-PGA and amino groups in the enzyme to obtain amide bond by the addition of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride. Outer membranes of polyurethane and polydimethylsiloxane (PDMS) were prepared by forming thin films using their solutions. The amperometric measurement were performed with a Potentiostat Model 3104 and examined at a potential of 0.6 V (vs. Ag/AgCl). The sensor response was measured at 40°C in a 0.1 mol/L phosphate buffer solution (PBS) of pH 7.4 containing 0.1 mol/L NaCl and human plasma containing 5.15 mmol/L glucose and 2.9 mmol/L lactate.

Good responses of glucose and lactate were observed in the measurement in PBS, and their correlation coefficient were r^2=0.9974±0.0010 (0-20 mmol/L) and r^2=0.9883±0.0100 (0-8 mmol/L), respectively. Moreover, similar response with that in PBS was observed also in human plasma. Since the cross-talking response currents were quite limited, individual responses of each sensor were successfully obtained.

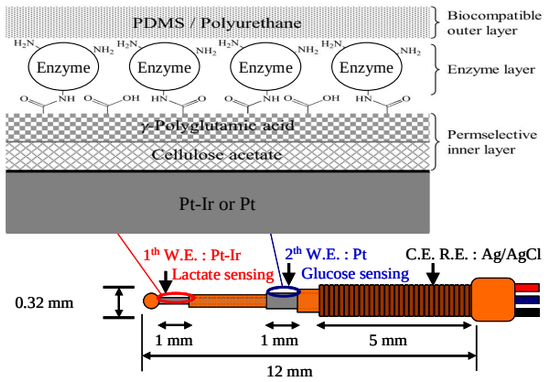
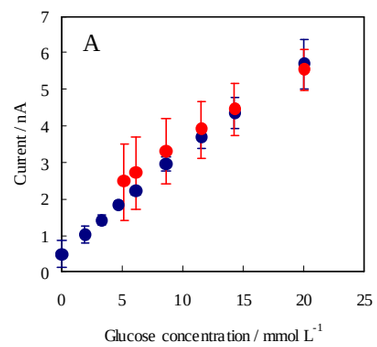


Fig. 1. Schematic illustration of a fine needle-type glucose lactate dual biosensor.



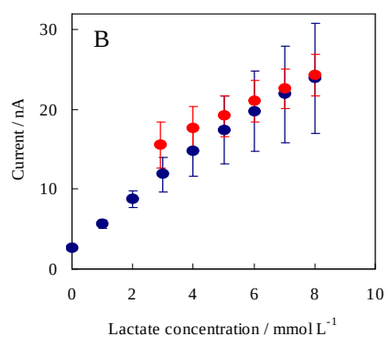


Fig. 2. Typical calibration curves of the glucose sensor (A) and lactate sensor (B) measured in PBS (blue circle) and human plasma (red circle) . *n* = 3.

References

[1] Yasuzawa M, Edagawa K, Matsunaga T, et al, *Anal. Sci.*, 27, 337-340 (2011).