HYBRID BIOSENSOR FOR UREA AND CREATININE
-WITH USE OF SUBSTITUTED POLYMETHYLGLUTAMATE-

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SUMMARY

Hybrid biosensor for urea and creatinine is based on a immobilized membrane and with immobilized nitrifying bacteria and an oxygen electrode. For chemical immobilization of enzyme, a substituted poly(γ-methyl-L-glutamate) membrane is used. The permeability of the membrane is controlled by the conditions used for membrane preparation. The urea sensor and creatinine sensor with the membrane exhibited a linear response over the range 2.5 - 25 mg dl^{-1}, and 1 - 10 mg dl^{-1} respectively.

1.INTRODUCTION

Creatinine and urea are very important diagnostic indices of kidney function, and biosensors for their determination which depend on potentiometric detection have been reported. A hybrid biosensor based on an amperometric method has also been described. A polymer based on partially transesterified poly(γ-methyl-L-glutamate) (PMG) for use as an enzyme carrier was developed. The modified polymer possesses functional groups that can react with enzyme amino groups in organic solvent(1).

$$P\text{-}(CH_2)_2COOCH_2CCl_3 + NH_2\text{-}ENZYME \longrightarrow P\text{-}(CH_2)_2CONH\text{-}ENZYME \quad (1)$$

This paper describes the immobilization of urease or creatininedeiminase onto the polymer, and membranes obtained are utilized in hybrid biosensor.

2.EXPERIMENTAL

2.1 Derivation of PMG

A highly reactive poly(γ-trichloroethylglutamate)(PTG) was prepared by transesterification of PMG with 2,2,2-trichloroethanol.

2.2 Preparation of immobilized enzyme membrane

Enzyme (creatininedeiminase or urease)(10mg) and 100mg of PTG were dissolved in 1 ml of dimethylsulfoxide(DMSO) solvent. The polymer and enzyme solution were mixed well and cast onto glass plate, then immediately immersed in 100 ml of 0.01M phosphate buffer, pH7.0, at a temperature above 10°C, for 1 h and the immobilized enzyme membrane was obtained.

2.3 Apparatus for hybrid biosensor

A hybrid biosensor consisted of an oxygen electrode, immobilized nitrifying bacteria, and an immobilized enzyme membrane, as shown in fig.1. The determination of urea or creatinine with the sensor was carried out with flow system at 30°C.

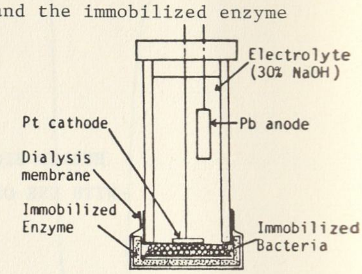

Fig. 1. Schematic diagram of Hybrid Biosensor

3.RESULTS

3.1 Immobilization condition

For the immobilization condition, the 5% concentration of PTG and 20°C of temperature during membrane preparation were chosed for the best condition.

3.2 Hybrid urea sensor

The concentration of urea measured by a flow injection method. Current decrease varied proportional to urea concentration, for PTG modified sensor, over the range 2.5 - 25 mg dl^{-1}. Although the detection limit only relates to an apparent urease concentration, it was considered as an index of enzyme activity and shown in table 1. The PTG modified sensor was more sensitive to urea than the sensor using polyvinylchloride(PVC) membrane.

3.3 Hybrid creatinine sensor

The characteristics of the sensor was shown in table 2. In previous study, creatininedeiminase was covalently immobilized onto a triamine membrane; the detection limit of the sensor was only 5 mg dl^{-1}, 5 times greater than this work. Comparison of two sensors is made in table 2. The reaction between the enzyme and triamine membrane took place on the surface of the membrane. In this work, the immobilization reaction proceeded in an organic solution of the enzyme and the polymer, so that the enzyme was more accesible to the functional groups on the polymer. Therefore more enzyme was immobilized on the PTG than on the triamine membrane.

Table 1 Comparison of hybrid urea sensor based on PVC and PTG membrane

Membrane	Response time	Linearity range	Stability*
PVC	2 min	5.0 - 50 mg dl^{-1}	50 %
PTG	1.5 min	2.5 - 25 mg dl^{-1}	73 %

Table 2 Comparison of hybrid creatinine sensor based on triamine and PTG membrane

Membrane	Response time	Linearity range	Stability*
Triamine	3 min	5 - 100 mg dl^{-1}	98 %
PTG	1 min	1 - 10 mg dl^{-1}	100 %

*activity after 2 weeks use