특별강연 II-2

Recognition of substrates by membrane potential

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1. INTRODUCTION

Recognition and binding of organic substrates by biological molecules are of vital importance in biophysics and biophysical chemistry. Most studies of the application focused on the development of biosensors, which detected reaction products generated by the binding between enzymes and substrates. Other types of biosensors in which membrane proteins (e.g., nicotinic acetylcholine receptor, auxin receptor ATPase, maltose bining protein, and glutamate receptor) were utilized as a receptor function were also developed.

In the previous study[1], the shifts in membrane potential, caused by the injection of substrates into a permeation cell, were measured using immobilized glucose oxidase membranes. It was suggested that the reaction product was not the origin of the potential shifts, but the changes in the charge density in the membrane due to the binding between the enzyme and the substrates generated the potential shifts.

In this study, γ-globulin was immobilized (entrapped) in a poly(γ-amino acid) network, and the shifts in the membrane potential caused by the injection of some amino acids were investigated.

2. THEORETICAL SHIFTS OF MEMBRANE POTENTIAL

The membrane potential has components from surface potential (Donnan potential) and the diffusion potential and is affected by shifts of charge density on the membrane surface. The shifts in membrane potential, caused by the injection of substrates into a permeation cell, were measured using immobilized enzyme membrane.

The shifts in the membrane potential caused by the injection of a substrate into a permeation cell is represented by

$$\triangle\triangle\Phi = \triangle\Phi(after) - \triangle\Phi(before)$$

where $\triangle \Phi(after)$ and $\triangle \Phi(before)$ are the membrane potential before and after the injection of substrate. The membrane potential is theoretically obtained by the Teorell -Meyer-Sievers(TMS) theory[2].

The shifts in membrane potential were considered to be generated by a change in the charge density in the enzyme membrane due to the binding of molecule to enzyme. Since the

membrane potential is a function of the charge density in a charged membrane, shifts in membrane potential can be generated by changes in the charge density in the enzyme membrane. A conformational change of the enzyme to bind the substrate may be the main reason for the change of the charge density in the enzyme membrane, since conformational change induces a change of charge density on enzyme surfaces.

This method leads to the measurements of binding (interaction) between host and guest molecules without any reaction such as occurs not only for enzyme/substrate but also for antibody/antigen and protein/specific ligand interactions. When an expensive enzyme or antibody is considered to be immobilized in the membrane, one might think that the enzyme or antibody should be conjugated or laminated on the surface of the membrane to reduce the amount of enzyme employed.

3. EXPERIMENTAL

Poly(γ-methyl-L-glutamate), PMLG, was purified by precipitation from 5 wt % dichloroethane in methanol. γ-globulin (IgG) was dissolved in a 1 wt % dichlorothane solution of PMLG. The casting solution used in this study had an IgG concentration of 4.0 mg L^{-1} PMLG solution. Immobilized (entrapped) IgG membranes were prepared by casting the IgG-PMLG solution onto flat Petri dished and then drying at room temperature for 6 days. The IgG-PMLG membranes were finally dried under vacuum at room temperature for 24 h and then stored at 10 °C.

Membrane potentials, $\triangle \Phi$, were measured as a function of the NaCl concentrations of the bulk phased by the same apparatus as described in previous work. The concentration of the aqueous NaCl solution was kept constant in one side of the chamber (side 1), C_1, at 1.0 x 10^{-3} mol dm^{-3} and was changed in the other side of the chamber (side 0), C_0, from 1.0 x 10^{-4}

mol dm^{-3} to 2.0 mol dm^{-3}. The potential was measured using a digital multimeter with Ag/AgCl electrodes at 37 ±0.02 °C.

After the pH in the cell registered a constant value (pH 5.71 ± 0.23), powders of the amino acid were carefully and quickly injected into the chamber of side 1. It takes less than 20 s for amino acids to be dissolved in the solution at *Cs* (concentration of injected substrate in the cell of side 1) ≤ 1.0 mol dm^{-3}. The shift in the membrane potential, caused by the injection of the amino acid into the cell, was monitored on a recorder, and the date were transferred to a PC.

4. RESULTS AND DISCUSSION

Changes in the membrane potential and pH upon the injection of D- and L-lysine at *Cs* = 0.01 mol dm^{-3} were measured for IgG-PMLG membranes. The results are shown in Figure 1. The shifts in membrane potential, $\triangle\triangle\phi$, defined by the difference in the potential before and after the injection of substrates in equilibrium were observed to be 50.41 ±2.0 mV (*n*=4) for D-lysine and 52.92 ±2.0 mV (*n*=4) for L-lysine. The same shifts in membrane potential were observed for D- and L-lysine. The pH shifts due to the injection of D- and L-lysine were observed to be 3.78 ±0.2 mV for D-lysine and 3.58 ±0.2 mV for L-lysine (Table 1). Although the initial membrane potential were negative (i.e., -36 ±2.0 mV), the membrane potential after the injection of lysine was observed to be a positive value (i.e., +15 ± 2.0 mV). The is due to the high isoelectric point of lysine (i.e., 9.70). Since the isolectric point of PMLG is approximately pH 6.0, the charge of PMLG at side 1 is positive before the injection of lysine and becomes negative after the injection of lysine. It is observed from the figure that the pH is quickly shifted, but the membrane potential is gradually changed when the substrate is injected into the cell.

Several substrates other than lysine were also investigated as substrates injected into the cell in this study. Figure 2 shows the changes in the membrane potential upon the injection of histidine, arginine, glutamic acid, and aspartic acid at *Cs* = 0.01 mol dm^{-3}. The potential response caused by the injection of each amino acid shows an individual and characteristic curve depending on the amino acid, and the difference in the potential curve between D-aspartic acid and L-aspartic acid is significantly observed in

IgG-PMLG membranes. $\Delta\Delta\Phi$ is observed to be positive when the isoelectric point of amino acids (i.e., histidine and arginine) is more than 6.0, and $\Delta\Delta\Phi$ is a negative value when the isoelectric point of amono acid (i.e., glutamic acid and aspartic acid) is less than 6.0. The absolute values of $\Delta\Delta\Phi$ for glutamic acid are found to be higher than those for aspartic acid, although the shifts in pH are observed to be the same for these amino acids. Although the shift in pH is a predominant factor in generating the shift in the membrane potential on the injection of amino acid into the cell, the characteristics of the amino acids also influence the $\Delta\Delta\Phi$ of each amino acid.

Table 1 summarizes the shifts in membrane potential, the shifts in pH and $t_{3/4}$ caused by the injection of various amino acids for the IgG membrane, where $t_{3/4}$ indicates the time at which 75% of the shift in $\Delta\Delta\Phi$ has been observed. The data presented in Table 1 are averages of four measurements, and the standard deviation of $t_{3/4}$ was calculated to be less than 0.40 min in this study.

The dependencies of the shifts in the membrane potential (Figure 3) and pH (Figure 4) on the concentration of amino acids injected into the dell were investigated. The shifts in membrane potential on the injection of L-lysine, L-arginine, and L-histidine increase with the increase in *Cs*, but the shifts in membrane potential on the injection of L-aspartic acid and L-glutamic acid decrease with the increase in *Cs* Figure 3. This id due to the difference in isoelectric points (IEP) of the amino acids (e.g., IEP > 6.0 for lysine, arginine, and histidine and IEP<6.0 for aspartic acid and glutamic acid) because the shifts in pH on the injection of lysine, arginine, and histidine increase with the increase in *Cs* and the shifts in pH on the injection of glutamic acid and aspartic acid decrease with the increase in *Cs*. Although the shift in pH is a predominant factor in determining the shift in the membrane potential on the injection of the amino acid into the cell, the $\Delta\Delta\Phi$ of L-glutamic acid is less than the $\Delta\Delta\Phi$ of L-aspartic acid at the same *Cs* and the shift in pH. The $\Delta\Delta\Phi$ of L-lysine is also found to be higher than the $\Delta\Delta\Phi$ of L-arginine at the same *Cs* and the same shift in pH. It is suggested that characteristics of amino acids influence the $\Delta\Delta\Phi$, and this is probably caused by the different binding site of each amino acid that induces a different conformation of IgG in the binding between IgG and the amino acid.

5. CONCLUSIONS

The shifts in membrane potential, caused by the injection of some substrates into a permeation cell, were measured using immobilized polymer membranes. The shifts in membrane potential were observed to be positive or negative when the isoelectric point of each amino acid injected into the cell was less or higher than 6.0. The potential response caused by the injection of each amino acid shows an individual and characteristic curve depending on the amino acid, and the difference in potential curves between D-aspartic acid and L-aspartic acid is significantly observed in the immobilized polymer membranes. The $t_{3/4}$ value was found to increase in the following order: lysine = glutamic acid < arginine < L-aspartic acid = asparagine < L-aspartic acid < histidine < alanaine, where $t_{3/4}$ indicates the time at which 75% of the shifts in membrane potential has been observed. The modified membrane potential theory provides satisfactory explanations for the membrane potential obtained experimentally before and after the injection of L-alanine, and the theoretical shift scan explain the experimental shifts in membrane potential due to the injection of L-alanine into the cell.

6. REFERENCES

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[2] H. U. Demisch and W. Pusch, *J.Colloid Interface Sci.*, **69** (1979) 247