Recognition of Amino Acids by Membrane Potential of Immobilized Globulin Membranes

K.-S. YUN,[1] T.-M. TAK,[1] S. SHIMADA,[2] T. NAKAGAWA,[2] M. HARA,[3] and A. HIGUCHI[3,*]

[1]Department of Natural Fiber Science, College of Agriculture & Life Science; Seoul National University, Suwon 440-744, Korea, [2]Department of Industrial Chemistry, School of Science and Technology, Meiji University, 1-1-1 Higashi-mita, Tama-ku, Kawasaki, Kanagawa 214, Japan, and [3]Department of Industrial Chemistry, Faculty of Engineering, Seikei University, 3 Kichijoji Kita-machi, Musashino, Tokyo 180, Japan

SYNOPSIS

The shifts in membrane potential, caused by the injection of some amino acids into a permeation cell, were measured using immobilized γ-globulin 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 γ-globulin membranes. The $t_{3/4}$ value was found to increase in the following order: lysine = glutamic acid < arginine < D-aspartic acid = asparagine < L-aspartic acid < histidine < alanine, 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 shifts can explain the experimental shifts in membrane potential due to the injection of L-alanine into the cell. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

Recognition and binding of organic substrates by biological molecules (i.e., enzymes, proteins, DNA, and RNA) are of vital importance in biophysics and biophysical chemistry. Most studies[1-8] for their 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,[9-13] auxin-receptor ATPase,[14] H^+/lactose cotransporter,[15] maltose binding protein,[16] and Na^+/D-glucose cotransporter[17]) were utilized as a receptor function were also developed.

In our previous studies,[18,19] 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.

The previous studies[18,19] prompted us to investigate the potential response of immobilized protein (except enzyme) membranes induced by specific substrates; this response does not generate a product. The shifts in membrane potential, caused by the injection of some amino acids into a permeation cell, were measured using immobilized serum albumin (BSA) membranes in our recent study.[20] It was suggested that the membrane potential theory provided a satisfactory explanation for the potential shifts obtained experimentally.

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

* To whom correspondence should be addressed.

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EXPERIMENTAL

Materials

Poly(γ-methyl-L-glutamate), PMLG, was kindly supplied by Ajinomoto Co., Inc. and purified by precipitation from 5 wt % dichloroethane in methanol. γ-globulin [Bovine, Cohn F-II (99%)] was purchased from Nakarai Tesque, Inc. (Kyoto, Japan). Other chemicals were of reagent grade and were used without further purification. Ultrapure water by the Toraypure LV-10T system (Toray Co., Ltd.) was used throughout the experiments.

Immobilized Protein Membranes

γ-globulin (IgG) was dissolved in a 1 wt % dichloroethane solution of PMLG. The casting solution used in this study had an IgG concentration of 4.0 mg cm^{-3} PMLG solution. Immobilized (entrapped) IgG membranes were prepared by casting the IgG–PMLG solution onto flat Petri dishes 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.

Measurement of Membrane Potential

Membrane potentials, $\Delta\phi$, were measured as a function of the NaCl concentrations of the bulk phases by the same apparatus as described in previous work.[18–20] The concentration of the aqueous NaCl solution was kept constant in one side of the chamber (side 1), C_1, at 1.0×10^{-3} mol dm^{-3} and was changed in the other side of the chamber (side 0), C_0, from 1.0×10^{-4} mol dm^{-3} to 2.0 mol dm^{-3}.[18–20] The potential was measured using a digital multimeter (range −99.9999 mV ~ +99.9999 mV, model 7561, Yokogawa Electronic Co.) with Ag/AgCl electrodes (TOA HS-205C, TOA Electronics Ltd.) at 37 ± 0.02°C.

The pH in the cell was also monitored with a pH meter (TOA HM-30S, TOA Electronics Ltd.). The membrane potential and its shift were measured when the pH in the cell was a constant value of ±0.01 after 20 min.

Measurement of Shifts in Membrane Potential

After the pH in the cell registered a constant value (pH 5.71 ± 0.23), powders of the amino acids 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 C_s (concentration of injected substrate in the cell of side 1) ≤ 0.1 mol dm^{-3}. The dissolving time and isoelectric point of the amino acids used in this study are shown in Table I.

The shift in the membrane potential, caused by the injection of the amino acid into the cell, was monitored on a recorder, and the data were transferred to a 16-bit personal computer (PC-9801VX, NEC Corp.). The shift in the pH was also monitored in this study.

The solution in the cell was replaced with ultrapure water several times after the measurements to

Table I Potential Changes and pH Changes Induced by the Injection of Amino Acids at C_0 = 0.1 mol dm^{-3}, C_1 = 1.0 mmol dm^{-3}, C_s = 0.01 mol dm^{-3} and 37°C

Amino Acid	MW	IEP	Soluble Time/min	pH (before)	pH (after)	ΔpH	Δφ (before)/mV	Δφ (after)/mV	ΔΔφ/mV	$t_{3/4}$/min
D-alanine	89.1	6.02	0.1	5.71	5.84	0.07	−32.82	−31.53	1.29	4.20
L-alanine	89.1	6.02	0.1	5.77	5.89	0.12	−35.01	−32.91	1.90	4.50
D-arginine	174.2	10.76	0.2	5.87	10.33	4.46	−32.00	15.77	47.77	1.45
L-arginine	174.2	10.76	0.2	5.80	10.38	4.58	−32.01	20.88	52.89	1.10
D-aspartic acid	133.1	2.77	0.1	5.80	3.11	−2.69	−32.30	−60.90	−28.60	1.70
L-aspartic acid	133.1	2.77	0.1	5.60	3.30	−2.30	−32.22	−55.75	−23.53	3.00
D-asparagine	132.1	5.41	0.1	5.70	5.32	−0.38	−34.80	−39.49	−4.69	1.60
L-asparagine	132.1	5.41	0.1	5.48	5.02	−0.46	−35.02	−43.03	−8.01	2.00
D-glutamic acid	147.1	3.22	0.2	5.71	3.30	−2.41	−32.10	−73.04	−40.94	0.35
L-glutamic acid	147.1	3.22	0.2	5.50	3.29	−2.21	−34.75	−68.66	−33.91	0.63
D-histidine	155.2	7.59	0.1	5.64	7.42	1.78	−36.27	2.19	38.46	3.85
L-histidine	155.2	7.59	0.1	5.68	7.48	1.80	−33.20	13.98	47.18	3.65
D-lysine	146.2	9.70	0.1	5.85	9.63	3.78	−35.31	15.10	50.41	0.95
L-lysine	146.2	9.70	0.1	5.83	9.41	3.58	−37.31	15.61	52.92	0.28

remove the residual amino acids. Each of the membranes can withstand measurements of more than 30 times over a period of one month. The membranes used in this study satisfied the condition that potential variation was within ±0.2 mV at a constant pH before the injection of substrates. The membrane potential shifts were reproducible to within ca. ±0.4 mV on repeated runs with the same membranes and ±2.0 mV on repeated runs with different membranes. Each point in Figures 3–6 and Table I is an average of 4 measurements (n = 4).

RESULTS AND DISCUSSION

Changes in Membrane Potential

Changes in the membrane potential and pH upon the injection of D- and L-lysine at C_s = 0.01 mol dm^{-3} were measured for IgG–PMLG membranes, where C_s is the concentration of the injected substrate in the cell of side 1. The results are shown in Figure 1. The shifts in membrane potential, $\Delta\Delta\phi$, 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-lysine and L-lysine. The pH shifts due to the injection of D- and L-lysine were observed to be 3.78 ± 0.2 (i.e., from pH 5.85 to pH 9.63) for D-lysine and 3.58 ± 0.2 for L-lysine (i.e., from pH 5.83 to pH 9.41, see Table I). Although the initial membrane potential was 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). This is due to the high isoelectric point of lysine (i.e., 9.70). Since the isoelectric 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 C_s = 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

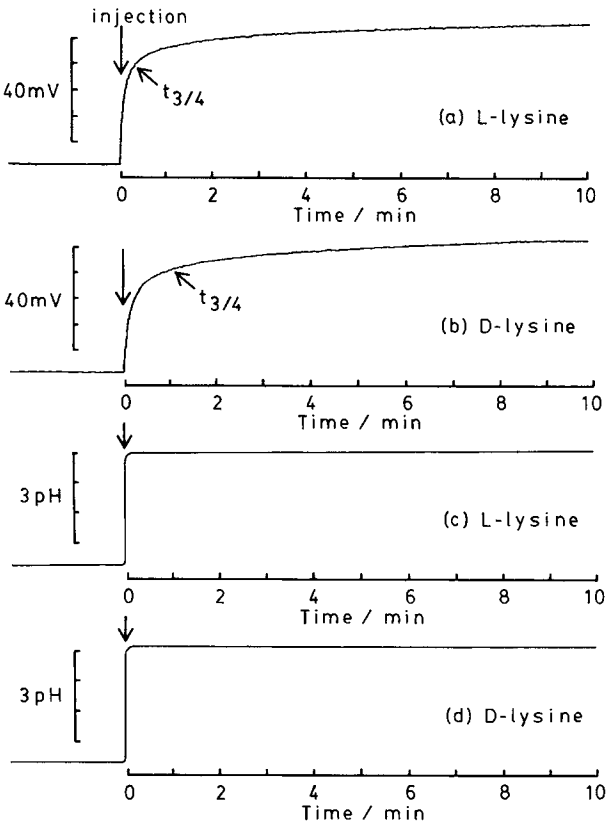

Figure 1 Time course of the membrane potential change (a, b) and the pH change (c, d) on the injection of L-lysine (a, c) and D-lysine (b, d) in the IgG–PMLG membrane at C_0 = 0.1 mol dm^{-3}, C_s = 0.01 mol dm^{-3}, C_1 = 1 mmol dm^{-3} and 37°C.

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 amino acids (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 I 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.[20] The data presented in Table I 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.

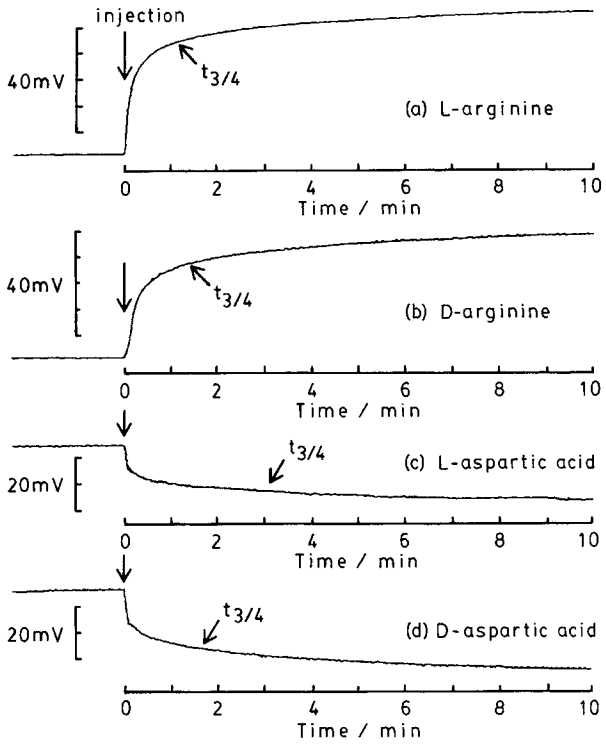

Figure 2-1 Time course of the membrane potential change on the injection of some amino acids [(a) L-arginine, (b) D-arginine, (c) L-aspartic acid, and (d) D-aspartic acid] in the IgG–PMLG membrane at C_0 = 0.1 mol dm^{-3}, C_s = 0.01 mol dm^{-3}, C_1 = 1 mmol dm^{-3} and 37°C.

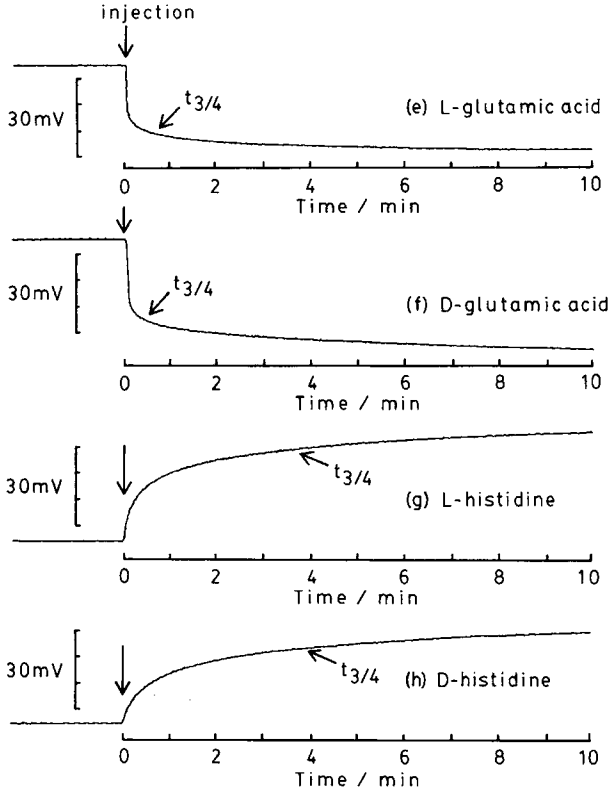

Figure 2-2 Time course of the membrane potential change on the injection of some amino acids [(e) L-glutamic acid, (f) D-glutamic acid, (g) L-histidine, and (h) D-histidine] in the IgG-PMLG membrane at C_0 = 0.1 mol dm^{-3}, C_s = 0.01 mol dm^{-3}, C_1 = 1 mmol dm^{-3} and 37°C.

Data for a nonsteady state ($t_{3/4}$ in this study) are sometimes good information for the recognition of substrates. We already reported that the concentration of multicomponent ions could be successfully estimated by analyzing the permeation of the ions through a poly(vinyl alcohol) membrane at the nonsteady state[21] and that $t_{3/4}$ in immobilized serum albumin membranes can be used to recognize several amino acids.[20] $t_{3/4}$ is found to show different values depending on each amino acid in the IgG–PMLG membranes and is observed to increase in the following order at C_s = 0.01 mol dm^{-3}, lysine = glutamic acid < arginine < D-aspartic acid = asparagine < L-aspartic acid < histidine < alanine. The $t_{3/4}$ of L-aspartic acid is found to be higher than the $t_{3/4}$ of D-aspartic acid. It is suggested that the parameter $t_{3/4}$ can recognize the isomer of aspartic acid injected into the cell. This probably originates from the different binding site of each isomer of amino acid in IgG.

From Table I, the results suggest that it may be possible to extract qualitative and quantitative information by combining $\Delta\Delta\phi$ and $t_{3/4}$ values to obtain a unique data pair that identifies a particular amino acid present at a certain concentration.

Concentration Dependence

The dependencies of the shifts in the membrane potential (Fig. 3) and pH (Fig. 4) on the concentration of L-lysine, L-arginine, L-histidine, L-glutamic acid, and L-aspartic acid injected into the cell were investigated and are shown in Figures 3 and 4. The shifts in membrane potential on the injection of L-lysine, L-arginine, and L-histidine increase with the increase in C_s, but the shifts in membrane potential on the injection of L-aspartic acid and L-glutamic acid decrease with the increase in C_s in Figure 3. This is 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 C_s and the shifts in pH

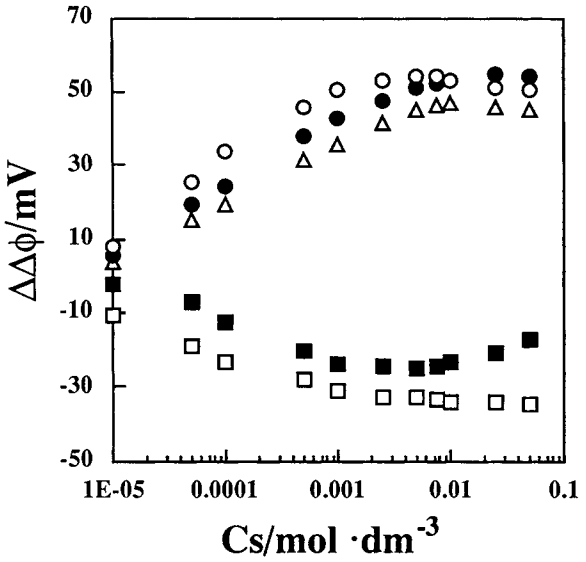

Figure 3 Concentration (C_s) dependence of shifts in the membrane potential for the IgG–PMLG membranes at C_0 = 0.1 mol dm^{-3}, C_1 = 1.0 mmol dm^{-3} and 37°C. The injected amino acids are (○) L-lysine, (●) L-arginine, (△) L-histidine, (□) L-glutamic acid, and (■) L-aspartic acid.

on the injection of glutamic acid and aspartic acid decrease with the increase in C_s. 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

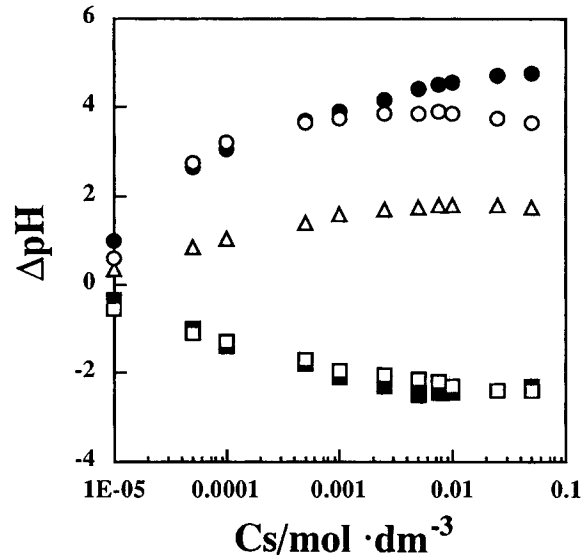

Figure 4 Concentration (C_s) dependence of pH in the cell of side 1 for the IgG–PMLG membranes at C_0 = 0.1 mol dm^{-3}, C_1 = 1.0 mmol dm^{-3} and 37°C. The injected amino acids are (○) L-lysine, (●) L-arginine, (△) L-histidine, (□) L-glutamic acid, and (■) L-aspartic acid.

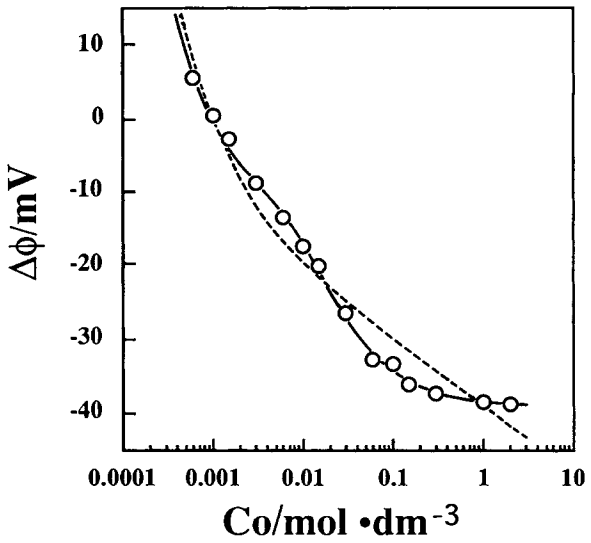

Figure 5 Membrane potential as a function of NaCl concentration, C_0, for the IgG–PMLG membrane before injection of (○) L-alanine at C_1 = 1.0 mmol mol^{-3} and 37°C. Broken line is calculated from the conventional TMS theory with C_x/K and U shown in Table II. Solid line is calculated from the modified TMS theory with C_x/K' and U shown in Table II.

acid is less than the $\Delta\Delta\phi$ of L-aspartic acid at the same C_s and the same 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 C_s 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.

Theoretical and Experimental Shifts

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

$$\Delta\Delta\phi = \Delta\phi(\text{after}) - \Delta\phi(\text{before}) \qquad (1)$$

where $\Delta\phi$(before) and $\Delta\phi$(after) are the membrane potentials before and after the injection of substrate. The membrane potential is theoretically obtained by the Teorell–Meyer–Sievers (TMS) theory:[22-24]

$$\Delta\phi = -\frac{RT}{ZF}\left[\ln\frac{C_1(1+4y_0^2)^{1/2}-\alpha}{C_0(1+4y_1^2)^{1/2}-\alpha} + U\ln\frac{(1+4\,y_1^2)^{1/2}-\alpha U}{(1+4\,y_0^2)^{1/2}-\alpha U}\right] \qquad (2)$$

Table II Membrane Potential Parameters for IgG–PMLG Membranes Before and After the Injection of L-Alanine

Membrane	C_s/M	C_xK^{-1}/mol dm^{-3}	U
Conventional TMS Theory			
IgG–PMLG	0.	9.73×10^{-4}	−0.146
IgG–PMLG	0.01	9.55×10^{-4}	−0.135
Modified TMS Theory			
IgG–PMLG	0.	$(24.9 + 93.4/[1 + 750C])^{-1}$	-1.19×10^{-2}
IgG–PMLG	0.01	$(26.7 + 100/[1 + 750C])^{-1}$	-7.81×10^{-3}

where $U = [\xi_+ - \xi_-]/[\xi_+ + \xi_-]$, ξ_+ and ξ_- are the mobilities of the cation and the anion, $y_0 = KC_0/C_x$, $y_1 = KC_1/C_x$, C_x is the effective fixed charge concentration, K is the thermodynamic partition coefficient,[11,15,19] α has a value of +1 or −1 when the membrane is positively or negatively charged, Z is the valence of the ion ($Z = 1$ in this study), and R, T, and F have the conventional meanings. Equation (2) indicates that the membrane potential is a function of the salt concentration (i.e., C_0 and C_1), C_x/K, α, and U.

We observed that $\Delta\Delta\phi$ on the injection of an amino acid having a lower isoelectric point than PMLG (i.e., aspartic acid and glutamic acid) is a negative value and that $\Delta\Delta\phi$ on the injection of an amino acid having a higher isoelectric point than PMLG (i.e., lysine and histidine) is a positive value. This tendency can be explained from $\Delta\phi$(after) having a positive α on the injection of aspartic acid and glutamic acid and a negative α on the injection of lysine, arginine, and histidine.

The membrane potential of the IgG membrane before and after the injection of L-alanine was measured as a function of C_0 at constant $C_1 = 10^{-3}$ mol dm^{-3} and is shown in Figure 5. Curve fitting of the experimental $\Delta\phi$ to Eq. (2) by adjusting C_x/K and U was performed by means of a nonlinear regression method (Marquardt method). Satisfactory fitting between experimental data and theoretical calculations was not, however, observed by using conventional TMS theory as shown in Figure 5.

One of the authors (A. H.) developed the modified TMS theory in which the concentration dependence of the partition coefficient was considered.[25] The theory could explain the membrane potentials of charged membranes having a lower water content. Since the water content of an IgG–PMLG membrane is only 0.16 water(g)/wet membrane(g), K' should be adequate instead of K in Eq. (2) as discussed in the modified TMS theory[25]:

$$K' = K_p + K_L S_L/(1 + K_L C) \quad (3)$$

where K_p is the Henry's law constant, S_L and K_L are the Langmuir-type capacity constant, and affinity constant and C is C_o or C_1, respectively.

Curve fitting of the experimental $\Delta\phi$ to Eq. (2) with Eq. (3) by adjusting C_x/K', and U was performed by means of the nonlinear regression method. The C_x/K and U values for the IgG–PMLG membrane thus obtained are summarized in Table II. It is found that $\Delta\phi$ calculated by the modified TMS theory satisfactorily explains the experimental $\Delta\phi$ before and after the injection of substrates (see Fig. 5). The theoretical shifts calculated by Eqs. (1)–(3) with C_x, K, and U found in Table II are plotted in Figure 6

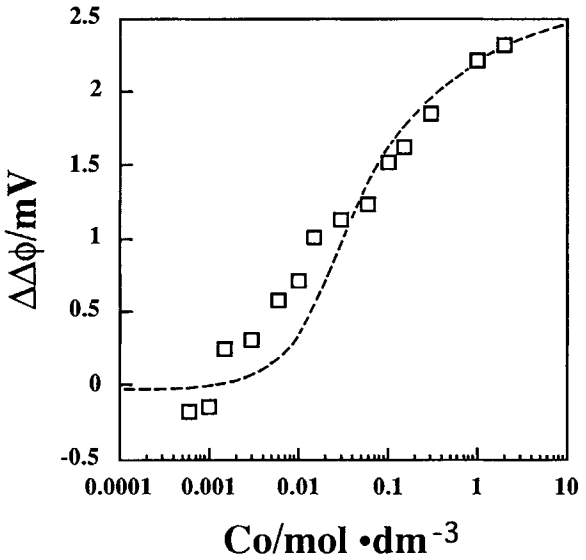

Figure 6 Shifts in the membrane potential as a function of C_0 for the IgG–PMLG membrane at $C_0 = 0.1$ mol dm^{-3}, $C_1 = 1.0$ mmol dm^{-3}, $C_s = 0.01$ mol dm^{-3} L-alanine and 37°C. Broken line is calculated from Eqs. (1)–(3) with C_x/K' and U shown in Table II.

together with $\Delta\Delta\phi$ obtained experimentally. The theoretical curve was found to explain the experimental shifts in membrane potential satisfactorily. The shifts in membrane potential due to the injection of substrate can be, therefore, explained by the modified TMS theory where C_x/K and U are the variable parameters before and after the injection of substrate.

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REFERENCES

1. K. Imai, T. Shiomi, K. Uchida, and M. Miya, *Biotechnol. Bioeng.*, **28,** 1721 (1986).
2. J. Janata and A. Bezegh, *Anal. Chem.*, **60,** 62 (1988).
3. T. Ikariyama, M. Furuki, and M. Aizawa, *Anal. Chem.*, **57,** 496 (1985).
4. F. F. Orlando, A. S. Ahmad, G. G. George, and J. L. Glenn, *Anal. Chem.*, **60,** 2397 (1988).
5. B. G. Gregg and A. Heller, *Anal. Chem.*, **62,** 258 (1990).
6. D. T. Pierce, P. R. Unwin, and A. J. Bard, *Anal. Chem.*, **64,** 1795 (1993).
7. L. Shufang and S. R. Walt, *Anal. Chem.*, **61,** 1069 (1989).
8. A. Higuchi and T. Nakagawa, *Bull. Chem. Soc. Jpn.*, **63,** 3209 (1990).
9. M. Gotoh, E. Tamiya, M. Momoi, Y. Kagawa, and I. Karube, *Anal. Lett.*, **20,** 857 (1987).
10. A. W. Dalziel, J. Georger, R. R. Price, A. Singh, and P. Yager, *Membrane Proteins, Proc. of the 1986 Membrane Protein Symposium,* S. C. Goheen, Ed., Bio-Rad Laboratory, Richmond, CA, 1987, p. 643.
11. M. E. Eldefrawi, S. M. Sherby, A. G. Andreou, N. A. Mansour, Z. Annau, N. A. Blum, and J. J. Valdes, *Anal. Lett.*, **21,** 1665 (1988).
12. R. F. Taylor, I. G. Marenchic, and E. J. Cook, *Anal. Chim. Acta,* **213,** 131 (1988).
13. K. R. Rogers, J. J. Valdes, and M. E. Eldefrawi, *Biosens. Bioelectron.*, **6,** 1 (1991).
14. M. Thompson, U. K. Krull, and M. A. Venis, *Biochem. Biophys. Res. Commun.*, **110,** 300 (1983).
15. H. Kiefer, B. Klee, E. John, Y.-D. Stierhof, and F. Jähnig, *Biosens. Bioelectron.*, **6,** 233 (1991).
16. L. Q. Zhou and A. E. Cass, *Biosens. Bioelectron.*, **6,** 445 (1991).
17. N. Sugao, M. Sugawara, H. Minami, M. Uto, and Y. Umezawa, *Anal. Chem.*, **65,** 363 (1993).
18. A. Higuchi, S. Ogawa, and T. Nakagawa, *J. Chem. Soc. Faraday Trans.*, **87,** 695 (1991).
19. A. Higuchi, S. Chida, and T. Nakagawa, *J. Chem. Soc. Faraday Trans.*, **87,** 2723 (1991).
20. A. Higuchi, Y. Ando, and T. Nakagawa, *Polym. J.*, **25,** 747 (1993).
21. A. Higuchi, T. Katoh, and T. Nakagawa, *J. Chem. Soc. Faraday Trans., I.*, **85,** 127 (1989).
22. H. U. Demisch and W. Pusch, *J. Colloid Interface Sci.*, **69,** 247 (1979).
23. T. Teorell, *Proc. Soc. Exp. Biol. Med.*, **33,** 282 (1935).
24. K. H. Meyer and J. F. Sievers, *Helv. Chim. Acta,* **19,** 649 (1936).
25. A. Higuchi and T. Iijima, *J. Appl. Polym. Sci.*, **31,** 419 (1986).

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