Recognition of Amino Acids by Membrane Potential and Circular Dichroism of Immobilized Albumin Membranes

MARIKO HARA,[1] MASAHIRO HIGUCHI,[2] NORIHIKO MINOURA,[2] and AKON HIGUCHI[1,*]

[1]Department of Industrial Chemistry, Faculty of Engineering, Seikei University, 3 Kichijoji Kita-machi, Musashino, Tokyo 180, Japan; [2]Department of Organic Materials, National Institute of Materials and Chemical Research 1-1 Higashi Tsukuba, Ibaraki 305, Japan

SYNOPSIS

The shifts in membrane potential, caused by the injection of some amino acids into a permeation cell, were measured using immobilized serum albumin membranes at isoelectric points of the amino acids. The effective fixed charge density was estimated to decrease after the injection of alanine, phenylalanine, and tryptophan and to increase after the injection of serine. The change in the fixed charge density originated from the conformational change of the immobilized albumin membranes induced by the binding between the albumin and amino acids in the membranes, since the conformational change of the immobilized albumin membranes induced by the binding of the amino acids to the serum albumin was observed from circular dichroism measurements. There was found, however, some discrepancy between the conformational change of the serum albumin in the albumin membranes detected by the membrane potential measurements and the circular dichroism measurements. This is explained by the fact that the circular dichroism measurements detect the increase or decrease in the α-helix, β sheet, and random coil contents; however, they do not always contribute to the detection of the change in the charge density due to the presence of the amino acid in the albumin membranes. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

The recognition and binding of organic substrates by biological macromolecules (i.e., enzymes, proteins, DNA, and RNA) are of vital importance in biophysical chemistry and analytical chemistry. Enzymes, proteins, DNA, and RNA are generally used as host molecules in the recognition in both living and artificial membranes. Most studies[1-9] of their application focused on the development of biosensors, which detected reaction products generated by the binding between the enzymes and substrates. Other types of biosensors have been developed in which membrane proteins (e.g., nicotinic acetylcholine receptor,[10-13] auxin-receptor ATPase,[14] H^+/lactose cotransporter,[15] maltose binding protein,[16] and Na^+/D-glucose cotransporter[17]) or DNA[18] were utilized as receptors. Aizawa et al.[19] developed an immunoresponsive membrane sensor for serological tests for syphilis, wherein the antibody concentration was determined by monitoring the membrane potential.

The membrane potential[20-27] has components from the surface potential (Donnan potential) and the diffusion potential and is affected by shifts in the charge density on the membrane surface. The shifts in the membrane potential, caused by the injection of substrates into a permeation cell, were measured using immobilized glucose oxidase,[28-30] albumin,[31] and globulin[32] membranes in our previous studies.[28-32] The shifts in the membrane potential upon the injection of both D- and L-glucose were clearly detected in the immobilized glucose oxidase membranes, because no pH change in the permeation cell was observed upon the injection of glucose into the cell.[28-30] In immobilized serum albumin membranes[31] and γ-globulin membranes,[32] the shift in the observed membrane potential was considered

* To whom correspondence should be addressed.

Journal of Applied Polymer Science, Vol. 58, 2025–2032 (1995)
© 1995 John Wiley & Sons, Inc. CCC 0021-8995/95/112025-08

to consist of shifts in the membrane potential originating from (a) pH changes in the cell and (b) changes in the fixed charge density in the membrane due to the binding between the amino acids and proteins. The pH change originated from the pH difference before and after the injection of amino acids into the cell. The pH in the cell after the injection of amino acids was found to be the isoelectric point (IEP) of the amino acids because of the buffering ability of the amino acids.[31,32]

In this study, bovine serum albumin 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 at the IEP of the amino acids used in the experiments. The shift in the membrane potential measured at the IEP is thought to originate solely from the changes in the fixed charge density in the membranes due to the binding between the amino acids and serum albumin, because no changes in the cell pH were expected upon the injection of amino acids into the cell. The conformational change of the membranes induced by the binding of amino acids to the serum albumin was also investigated based on circular dichroism measurements and is compared with the results obtained from the shifts in the membrane potential.

EXPERIMENTAL

Materials

Poly(γ-methyl-L-glutamate) (PMLG) was kindly supplied by Ajinomoto Co. and purified by precipitation from 5 wt % dichloroethane in methanol. Bovine serum albumin (CRG-7, fatty acid-free type) (BSA) was purchased from Nakarai Tesque (Kyoto, Japan). Other chemicals were of reagent grade and were used without further purification. Ultrapure water was used throughout the experiments.

Immobilized Protein Membranes

BSA was dissolved in a 1 wt % dichloroethane solution of PMLG. The casting solution used in this study had a BSA concentration of 4.0 mg cm^{-3} of PMLG solution. Immobilized (entrapped) BSA membranes were prepared by casting the BSA–PMLG solution onto flat Petri dishes and then air-drying at room temperature for 6 days. The BSA–PMLG membranes were finally dried under vacuum at room temperature for 24 h and then stored at 10°C.

Measurement of Membrane Potential

The membrane potential, $\Delta\phi$, was measured in cells that consisted of two chambers separated by the protein membranes as described in a previous article.[28-32] The concentrations of the aqueous NaCl solutions were 1.0×10^{-3} mol dm^{-3} (M) in one side of the chamber (side 1), C_1, and 0.1M in the other side of the chamber (side 0), C_0. The potential was measured using a digital multimeter (range −99.9999–+99.9999 mV, Model 7561, Yokogawa Electronic Co.) with Ag/AgCl electrodes (TOA HS-205C, TOA Electronics) at 37 ± 0.02°C.

The pH in the cell was monitored with a pH meter (TOA HM-30S, TOA Electronics) and was adjusted to the IEP of the amino acids (IEP of alanine = 6.02, phenylalanine = 5.48, tryptophan = 5.89, and serine = 5.68) with the introduction of 0.01M NaOH solution into the cells. The membrane potential and its shift were measured when the pH in the cell reflected the IEP of the injected amino acids based on a pH variation of ±0.01 for 20 min.

Measurement of Shifts in the Membrane Potential

After the pH in the cell registered the IEP of the amino acid injected, powders of the amino acids were carefully and quickly injected into the chamber of side 1.[31,32] Less than 20 s was needed for the amino acids to dissolve in the solution at Cs (the concentration of injected substrate in the cell of side 1) $< 0.1M$.[32]

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 32-bit personal computer (PC-9801BX, NEC Corp.). The shift in the pH was also monitored during this study.

The solution in the cell was replaced with ultrapure water several times after each set of measurements to remove the residual amino acids. Each of the membranes are capable of withstanding more than 30 measurements over a 1 month period.[30,31] 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.[31,32] Each point in Table I is an average of four measurements ($n = 4$). Figures 1–8 show typical curves which were selected from four measurements performed under the same conditions.

CIRCULAR DICHROISM OF MEMBRANES

Thin membranes (i.e., 1–3 μm thick) were prepared by casting the BSA–PMLG solution and PMLG solution on quartz plates ($6 \times 0.99 \times 0.125$ cm). The membrane loaded on the quartz plate was immersed in either the phosphate buffer solution at the IEP of the amino acid or the $0.01M$ amino acid + phosphate buffer solution at the IEP of the amino acid. The circular dichroism (CD) of the membrane loaded on the quartz plate was measured with a JASCO J-600 instrument (JEOL) after the membrane was immersed in the solution for 30 min prior to the measurements.

RESULTS AND DISCUSSION

Shifts in the Membrane Potential

The changes in the membrane potential upon the injection of D-alanine, L-alanine, D-phenylalanine, L-phenylalanine, D-tryptophan, L-tryptophan, D-serine, and L-serine were measured for the BSA–PMLG membranes at $Cs = 0.01M$ and at the isoelectric point of each amino acid, where Cs is the concentration of the injected amino acid in the cell of side 1. The time courses of the membrane potential change are shown in Figures 1 and 2. Changes in the pH after the injection of the amino acids were not observed within ±0.01 in this study because the pH in the cell was adjusted to the IEP of each amino acid.

The membrane potential before the injection of various amino acids, $\Delta\phi$(before), was observed to be -19 ± 2 mV at $C_1 = 10^{-3}\ M$ and $Co = 10^{-1}\ M$. The membrane potential after the injection of substrates, $\Delta\phi$(after), was found to be higher than $\Delta\phi$(before) for the injections of alanine, phenylalanine, and tryptophan. On the contrary, after the injection of serine $\Delta\phi$(after) was found to be less than $\Delta\phi$(before).

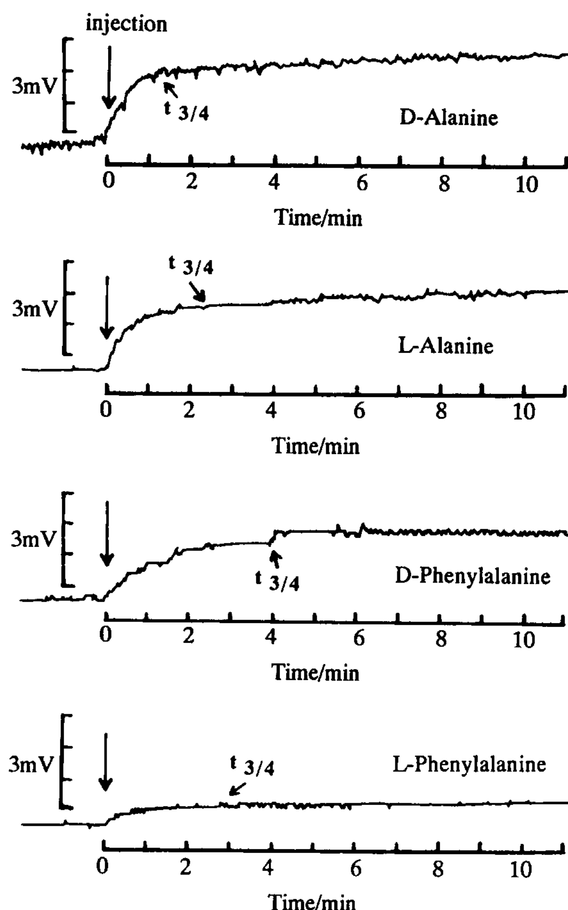

Figure 1 Time course of the membrane potential change after the injection of amino acids at 37°C.

The shifts in the membrane potential, $\Delta\Delta\phi$, defined by the difference in the potential before and after the injection of the substrates in equilibrium [i.e., eq. (1)] were found to be 0.0 ± 0.5 mV for the

Table I Shifts in Membrane Potential, $t_{3/4}$, and Shifts in Ellipticity of CD Spectra Induced by Various Amino Acids in Immobolized BSA Membranes

Substrate	$\Delta\Delta\phi$ (mV)	$t_{3/4}$ (min)	$\Delta\theta_{208}$ (%)	$\Delta\theta_{222}$ (%)
D-Alanine	3.36	1.3	−5.65	−5.30
L-Alanine	2.85	2.2	5.88	4.42
D-Serine	−2.27	0.3	−7.52	−3.89
L-Serine	−2.54	0.4	4.29	9.82
D-Tryptophan	1.55	3.2	16.49	−12.36
L-Tryptophan	1.03	3.0	7.49	19.71
D-Phenylalanine	2.42	3.9		
L-Phenylalanine	0.72	2.7		

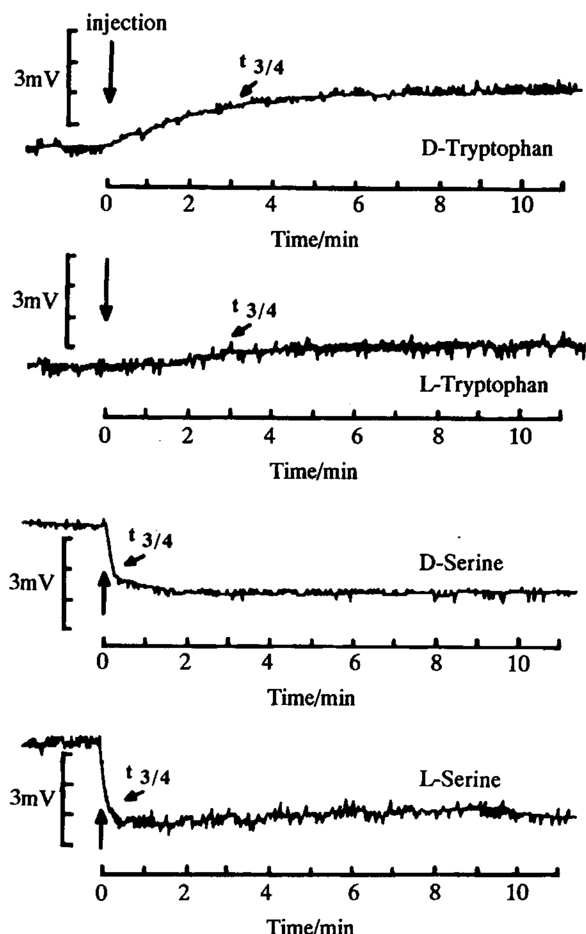

Figure 2 Time course of the membrane potential change after the injection of amino acids at 37°C.

PMLG membranes and −2.5–3.4 mV for the BSA–PMLG membranes depending upon the amino acids injected:

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

Table I summarizes the $\Delta\Delta\phi$ for the BSA–PMLG membranes caused by the injection of various amino acids. The data presented in Table I are averages of four measurements and the standard deviation of $\Delta\Delta\phi$ is calculated to be less than 0.5 mV in this study. The $\Delta\Delta\phi$ were observed to increase in the following order at $Cs = 0.01M$, serine < 0 mV < L-phenylalanine = L-tryptophan < D-tryptophan < D-phenylalanine < L-alanine < D-alanine. It was observed that $\Delta\Delta\phi$ after the injection of the D-isomer was higher than that of the corresponding L-isomer for alanine, phenylalanine, and tryptophan in this study. Similar results were also observed in a previous study[31] where the $\Delta\Delta\phi$ was estimated from the apparent shift in the membrane potential minus the shift in the membrane potential originating from the pH change in the cell. The $\Delta\Delta\phi$ (= $\Delta\Delta\phi_{int}$ expressed in the previous study[31]) of D-alanine was reported to be higher than that of L-alanine, and the $\Delta\Delta\phi$ of D-tryptophan was higher than that of L-tryptophan in the previous study.[31]

D- and L-Serine produced negative $\Delta\Delta\phi$ values in this study (see Table I), although $\Delta\Delta\phi_{int}$ of D- and L-serine was observed to show positive values (i.e., 1.5 and 1.9 mV) in the previous study.[31] The intrinsic shift in the membrane potential, $\Delta\Delta\phi_{int}$, caused by the binding of the substrate to BSA, was obtained from the following calculation in the previous study[31]:

$$\Delta\Delta\phi_{int} = \Delta\phi(\text{after}) - \Delta\phi(N_2\ \text{bubbling}) \qquad (2)$$

where $\Delta\phi$(after) is $\Delta\phi$ after the injection of substrate and $\Delta\phi(N_2$ bubbling) is $\Delta\phi$ before the injection of substrate on the same pH after the injection of substrate which is obtained under N_2 bubbling.[31] The previous method to obtain $\Delta\Delta\phi$ (= $\Delta\Delta\phi_{int}$) needs the measurements of not only $\Delta\phi$(after) but also $\Delta\phi(N_2$ bubbling). The present method, the mea-

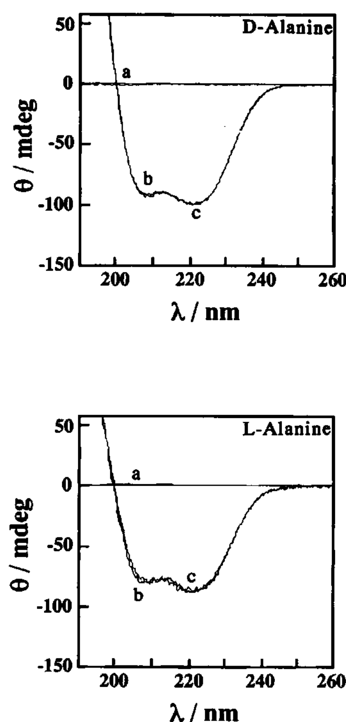

Figure 3 CD spectra of (a) 0.01M alanine solution, (b) PMLG membranes in phosphate buffer, and (c) PMLG membranes in 0.01M alanine + phosphate buffer solution.

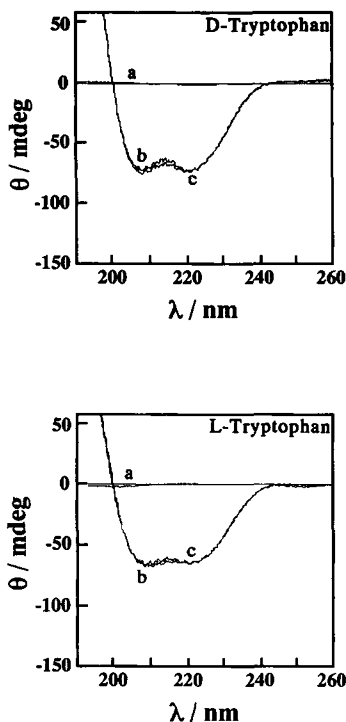

Figure 4 CD spectra of (a) 0.01M tryptophan solution, (b) PMLG membranes in phosphate buffer, and (c) PMLG membranes in 0.01M tryptophan + phosphate buffer solution.

surements of $\Delta\Delta\phi$ at the IEP, do not need to obtain calibration curves of $\Delta\Delta\phi$ vs. pH shift for the estimation of $\Delta\phi(N_2$ bubbling) and especially contributes to give more reliable results measured for the small $\Delta\Delta\phi$ values such as $\Delta\Delta\phi$ of serine.

The nonsteady-state parameter, $t_{3/4}$, was used for the recognition of substrates in the previous study.[31,32] $t_{3/4}$ indicates the time which shows 75% of the shift of $\Delta\Delta\phi_{\text{app}}$. $\Delta\Delta\phi_{\text{app}}$ used in the previous study[31] consists of the potential shifts originated from (a) pH changes in the cell and membrane and (b) changes in the fixed charge density in the membrane due to the binding between the amino acids and proteins. $t_{3/4}$ obtained from the time which shows 75% of $\Delta\Delta\phi$ at the IEP of amino acids is also obtained in this study. Table I also summarizes $t_{3/4}$ caused by the injection of some amino acids in the BSA–PMLG membranes at $Cs = 0.01M$. It is found in Table I that $t_{3/4}$ increases in the following order at $Cs = 0.01M$: serine < alanine < tryptophan = phenylalanine.

The $t_{3/4}$ is found to show the characteristics of each amino acid. $t_{3/4}$ values obtained in this study do not completely agree with $t_{3/4}$ values reported in the previous study.[31] This is because $t_{3/4}$ is measured at the IEP of the amino acid in this study, and $t_{3/4}$ obtained in the previous study[31] contains the time shift of membrane potential originated from the pH changes in the cell and the BSA–PMLG membranes.

The membrane potential is generally expressed by the Teorell–Meyer–Sievers (TMS) theory[20–27]:

$$\Delta\phi = -\frac{RT}{zF}\left\{\ln\frac{C_1[1+4y_0^2]^{1/2}-\alpha}{C_0[1+4y_1^2]^{1/2}-\alpha} + U\cdot\ln\frac{[1+4y_1^2]^{1/2}-\alpha U}{[1+4y_0^2]^{1/2}-\alpha U}\right\} \quad (3)$$

where $U = [\xi_+ - \xi_-]/[\xi_+ + \xi_-]$; ξ_+ and ξ_- are the mobilities of the cation and the anion, respectively; $y_0 = KC_0/Cx$; $y_1 = KC_1/Cx$; Cx is the effective fixed charge concentration[20–27]; K is the thermodynamic partition coefficient[20–27]; α has a value of +1 or −1 when the membrane is positively or negatively charged ($\alpha = +1$ in this study); z is the valence of the ion ($z = 1$ in this study); and R, T, and F have their conventional meanings. The only unknown parameters in eq. (3) are Cx/K and U.

Cx/K and U before the injection of the amino acid at the IEP of L-alanine were estimated to be

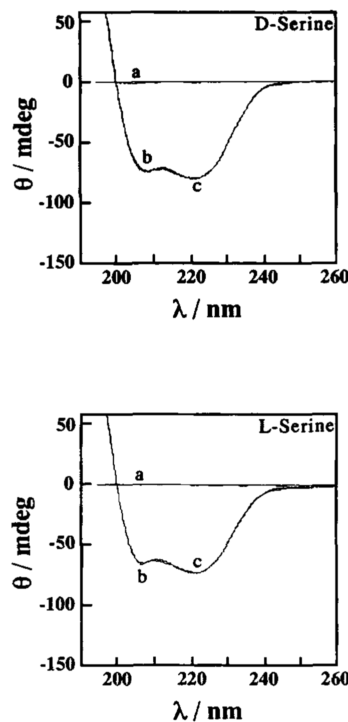

Figure 5 CD spectra of (a) 0.01M serine solution, (b) PMLG membranes in phosphate buffer, and (c) PMLG membranes in 0.01M serine + phosphate buffer solution.

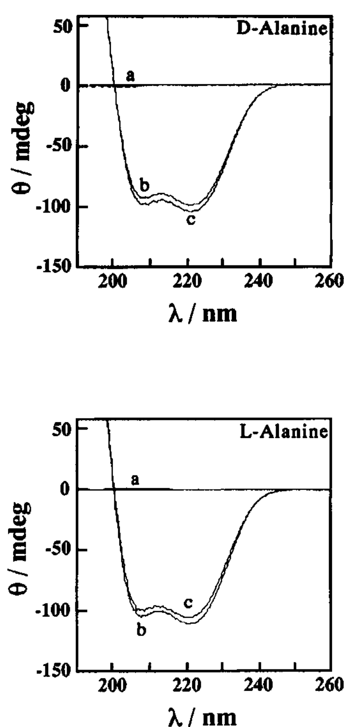

Figure 6 CD spectra of (a) 0.01*M* alanine solution, (b) BSA–PMLG membranes in phosphate buffer, and (c) BSA–PMLG membranes in 0.01*M* alanine + phosphate buffer solution.

0.24 m*M* and 0.0638 from the previous study.[31] It is known that Cx/K is the predominant influence on the membrane potential; therefore, the positive values of the $\Delta\Delta\phi$ are explained by the fact that the effective fixed charge density decreases after the injection of alanine, phenylalanine, and tryptophan based on the estimation from eq. (3), whereas the negative values of $\Delta\Delta\phi$ can be attributed to the fact that the effective fixed charge density increases after the injection of serine based on the estimation from eq. (3).

We suggest that the increase or decrease in the effective fixed charge density after the injection of amino acids originates from the conformational change in the BSA–PMLG membranes induced by the binding between the amino acids and the BSA. Therefore, the circular dichroism of the BSA–PMLG membranes was investigated to determine whether the conformational change in the BSA–PMLG membranes was induced by the existence of guest molecules, the amino acids.

Circular Dichroism of Membranes

The circular dichroism (CD) of the PMLG membranes in the phosphate buffer solution and the 0.01*M* amino acid (alanine, tryptophan, or serine) + phosphate buffer solution was measured before the investigation of the CD spectra of the BSA–PMLG membranes at the IEP of the amino acids and is shown in Figures 3–5. Dual peaks around 208 and 222 nm, which mainly indicate an α-helix conformation,[33–35] were observed in the CD spectra. Exactly the same spectra were obtained when the CD spectra of the PMLG membranes immersed in the buffer solution were compared with the CD spectra of PMLG membranes immersed in the amino acid + phosphate buffer solution. The conformational change of the PMLG membranes induced by the sorption of the amino acid could not be detected in the CD measurements in this study.

The circular dichroism of BSA–PMLG membranes in the phosphate buffer solution and 0.01*M* amino acid (alanine, tryptophan, or serine) + phosphate buffer solution at the IEP of the amino acid was measured and is shown in Figures 6–8. Dual peaks around 208 and 222 nm were also observed in the CD spectra. The BSA–PMLG membranes in the phosphate buffer solution show spectra similar to those of the PMLG membranes. This is because both the PMLG and BSA are polypeptides, and it is

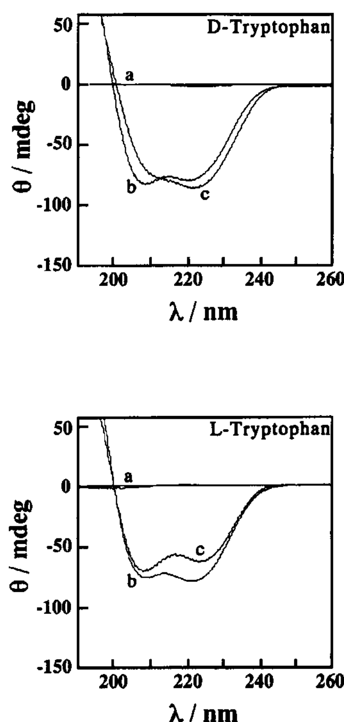

Figure 7 CD spectra of (a) 0.01*M* tryptophan solution, (b) BSA–PMLG membranes in phosphate buffer, and (c) BSA–PMLG membranes in 0.01*M* tryptophan + phosphate buffer solution.

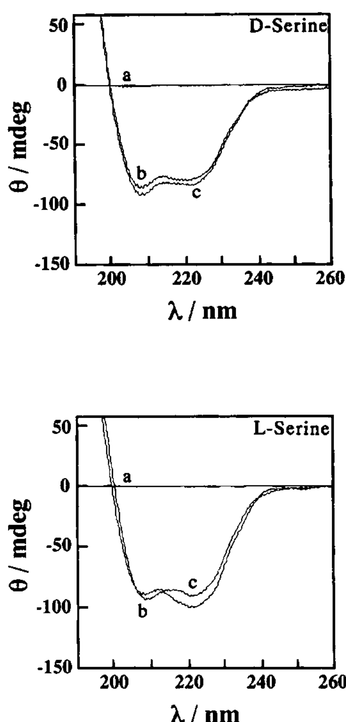

Figure 8 CD spectra of (a) $0.01M$ serine solution, (b) BSA–PMLG membranes in phosphate buffer, and (c) BSA–PMLG membranes in $0.01M$ serine + phosphate buffer solution.

known that they have mainly an α-helix conformation. In this study, a significant difference was observed between the CD spectra of BSA–PMLG membranes immersed in the buffer solution and the amino acid solution. The most significant difference was observed when the BSA–PMLG membranes were immersed in the tryptophan solution (see Fig. 7). The difference in the CD spectra can be explained by the conformational change in BSA caused by the binding to amino acids in the BSA–PMLG membranes, because the difference in the CD spectra was not observed in the PMLG membranes.

The shifts in the ellipticity of the CD spectra, $\Delta\theta_{208}$ and $\Delta\theta_{222}$, are defined in this study as

$$\Delta\theta_{208} = 100 \cdot [\theta_{208}(\text{amino acid}) - \theta_{208}(\text{buffer})]/\theta_{208}(\text{buffer}) \quad (4)$$

$$\Delta\theta_{222} = 100 \cdot [\theta_{222}(\text{amino acid}) - \theta_{222}(\text{buffer})]/\theta_{222}(\text{buffer}) \quad (5)$$

where θ_{208} (buffer) and θ_{222} (buffer) are the ellipticity of the BSA–PMLG membranes in the phosphate buffer solution at $\lambda = 208$ nm and $\lambda = 222$ nm, and θ_{208} (amino acid) and θ_{222} (amino acid) are the ellipticity of the BSA–PMLG membranes in the amino acid solution at $\lambda = 208$ nm and $\lambda = 222$ nm. $\Delta\theta_{208}$ and $\Delta\theta_{222}$ for D,L-alanine, D,L-serine, and D,L-tryptophan are summarized in Table I. The values for $\Delta\theta_{208}$ and $\Delta\theta_{222}$ presented in Table I are the averages of four measurements, and the standard deviation of $\Delta\theta_{208}$ and $\Delta\theta_{222}$ is calculated to be less than 1.2% in this study. The values of $\Delta\theta_{208}$ and $\Delta\theta_{222}$ for L-alanine, L-serine, and L-tryptophan are positive, which indicates that the content of the α-helix conformation decreases when the L-isomer binds to BSA, whereas the values of $\Delta\theta_{208}$ and $\Delta\theta_{222}$ for D-alanine and D-serine are negative, which indicates that the content of the α-helix conformation increases when the D-isomer binds to BSA. The values of $\Delta\theta_{208}$ and $\Delta\theta_{222}$ for D-tryptophan showed a rather unique tendency compared with the values of $\Delta\theta_{208}$ and $\Delta\theta_{222}$ for another D-isomer. The value of $\Delta\theta_{208}$ for D-tryptophan was found to be positive, whereas the value for $\Delta\theta_{222}$ was negative, and the CD spectrum of the BSA–PMLG membrane immersed in the amino acid solution intersected the CD spectrum in the buffer solution.

The values of $|\Delta\theta_{208}|$ and $|\Delta\theta_{222}|$ for tryptophan were found to be greater than those for alanine and serine, when the BSA–PMLG membranes were immersed in the $0.01M$ amino acid + buffer solution, but the value for $|\Delta\Delta\phi|$ of serine or alanine was higher than the value of $|\Delta\Delta\phi|$ of tryptophan at $Cs = 0.01M$. The values of $\Delta\theta_{208}$ and $\Delta\theta_{222}$ for D-alanine and D-serine show the opposite sign of the values of $\Delta\theta_{208}$ and $\Delta\theta_{222}$ for L-alanine and L-serine, but the sign of the value of $\Delta\Delta\phi$ for the L-isomer is the same sign as that for $\Delta\Delta\phi$ for the corresponding D-isomer. Therefore, some discrepancy was found between the conformational change in BSA in the BSA–PMLG membranes detected by the membrane potential measurements and CD measurements in this study, although the conformational change of the BSA–PMLG membranes induced by the binding between BSA and amino acids was detected by both the membrane potential measurements and the CD measurements. This is explained by the fact that the CD measurements can detect an increase or decrease in the α-helix, β sheet, and random coil contents but do not always contribute to the detection of the change in the charge density due to the presence of amino acids in the BSA–PMLG membranes.

We are grateful to Dr. Y. Miyachi (Ajinomoto Co. Inc.) for the gift of the PMLG solution. This research was partially supported by the Asahi Glass Foundation.

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. Y. 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. A. 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. Demura and T. Asakura, *J. Membr. Sci.,* **59,** 39 (1991).
10. M. Gotoh, E. Tamiya, M. Momoi, Y. Kagawa, and I. Karube, *Anal. Lett.,* **20,** 857 (1987).
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. J. Krull, and M. A. Venis, *Biochem. Biophys. Res. Commun.,* **110,** 300 (1983).
15. H. Kiefer, B. Klee, E. John, Y.-D. Stierhof, and F. Jahnig, *Biosens. Bioelectron.,* **6,** 233 (1991).
16. L. Q. Zhou and A. E. G. Cass, *Biosens. Bioelectron.,* **6,** 445 (1991).
17. N. Sugao, M. Sugawara, H. Minami, M. Uto, and Y. Umezawa, *Anal. Chem.,* **65,** 363 (1993).
18. Y. Okahata, Y. Matsunobu, K. Ijiro, M. Mukae A. Murakami, and K. Makino, *J. Am. Chem. Soc.,* **114,** 8299 (1992).
19. M. Aizawa, S. Kato, S. Suzuki, Y. Nagamura, R. Shinohara, and I. Ishiguro, *Kobunshi Ronbunshu* (in Japanese), **34,** 813 (1977).
20. H. U. Demisch and W. Pusch, *J. Colloid Interface Sci.,* **69,** 247 (1979).
21. A. Higuchi and T. Iijima, *J. Appl. Polym. Sci.,* **31,** 419 (1986).
22. A. Higuchi and T. Nakagawa, *J. Membr. Sci.,* **32,** 267 (1987).
23. A. Higuchi and T. Nakagawa, *J. Chem. Soc. Faraday Trans. I,* **85,** 3609 (1989).
24. M. Higa, A. Tanioka, and K. Miyasaka, *J. Membr. Sci.,* **49,** 145 (1990).
25. T. Kinoshita, T. Yamashita, T. Iwata, A. Takizawa, and Y. Tsujita, *J. Macromol. Sci. Phys. B,* **22,** 1 (1983).
26. T. Teorell, *Proc. Soc. Exp. Biol. Med.,* **33,** 282 (1935).
27. K. H. Meyer and J. F. Sievers, *Helv. Chim. Acta,* **19,** 649 (1936).
28. A. Higuchi, S. Ogawa, and T. Nakagawa, *J. Chem. Soc. Faraday Trans.,* **87,** 695 (1991).
29. A. Higuchi, S. Chida, and T. Nakagawa, *J. Chem. Soc. Faraday Trans.,* **87,** 2723 (1991).
30. A. Higuchi, M. Hara, K.-S. Yun, and T.-M. Tak, *J. Appl. Polym. Sci.,* **51,** 1735 (1994).
31. A. Higuchi, Y. Ando, and T. Nakagawa, *Polym. J.,* **25,** 747 (1993).
32. K.-S. Yun, T.-M. Tak, S. Shimada, T. Nakagawa, M. Hara, and A. Higuchi, *J. Appl. Polym. Sci.,* **55,** 343 (1995).
33. N. Greenfield and G. D. Fasman, *Biochemistry,* **8,** 4108 (1969).
34. N. S. Simmons, C. Chohen, A. G. Szent-Gyorgyi, O. B. Wetlaufer, and E. R. Blout, *J. Am. Chem. Soc.,* **83,** 4766 (1961).
35. E. H. Strickland, J. Horwitz, and C. Billups, *Biochemistry,* **8,** 3205 (1969).

Received January 17, 1995
Accepted April 23, 1995