Solute Permeability Enhancement at a Specific pH by an Amphiphilic Copolypeptide Membrane

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ABSTRACT: pH-responsive polypeptide, poly(Leu-Gln($EtNH_2$)), membranes consisting of hydrophobic leucine (Leu) and hydrophilic N^{ω}-(β-(aminoethyl)-L-glutamine) (Gln($EtNH_2$)) residues have been prepared and pH-induced conformational changes of the polypeptide in membranes and their relation to membrane swelling and solute permeation properties were investigated. IR spectra of the membrane containing 31% Gln($EtNH_2$) residues indicated an α-helix to random coil transition by decreasing pH with the midpoint of the transition at ca. pH 4.7. Moreover, fluorescence measurements of *N*-phenyl-1-naphthylamine entrapped in the membrane showed that a strong association between the hydrophobic leucine residues could be formed when the conformation of the polypeptide was converted from a rigid α-helix to a flexible charged coil below the midpoint pH of the transition. On the other hand, the degree of hydration of the poly(Leu-Gln($EtNH_2$)) membranes with various Gln($EtNH_2$) content was steeply increased with decreasing pH from the alkaline to weak acid pH values, resulting from the conformational transition following an ionization of Gln($EtNH_2$) residues. However, the further decrease in pH induced a remarkable deswelling of the membranes, which could be explained in terms of the formation of hydrophobic clusters between leucine moieties in the induced charged coil conformation yielding intermolecular bridges in the membranes. The styrene glycol permeabilities and/or diffusibilities through the membrane with 31% Gln($EtNH_2$) residues, as a result, could be effectively regulated by the pH-induced swelling and deswelling of the membrane; i.e., the permeability of the solute could be significantly enhanced only at around pH 4.5. It is also found that, similar to the case of the membrane system, the solute release rate from a capsule membrane of poly(Leu-Gln($EtNH_2$)) containing 22% Gln($EtNH_2$) residues could be reversibly controlled by the external pH.

Introduction

Biological membranes sense their environment and make responses to a variety of environmental stimuli. To develop novel membrane systems based on the stimulus-response coupling of biological membranes may be important in diverse fields such as medical, pharmaceutical, foregoing information transfer systems, and so on.

Recently, attention has been paid for mimicking chemo- and photosensory transduction systems whose main concern is the appropriate regulation of membrane functions by environmental changes such as chemicals,[1-6] pH,[7-15] and light[16-20] via conformational changes of the membrane molecules. On the other hand, a fundamental approach to the stimuli-induced phase change of gels has been developed.[21,22]

Because of a requirement of efficient transport and/or localization of drugs to the intestines and tumor cells, increasing attention has been still focused on pH-responsive membranes.[23-25] These studies showed that polyelectrolytes in the membrane are particularly useful for sensoring external pH. We have also reported that transport properties of polypeptide membranes can be effectively controlled by pH-induced α-helix to coil transition of ionizable polypeptides as an on/off switching unit in the membranes.[7-9] It may be said, therefore, that permeability of a membrane can be enhanced (reduced) above or below the boundary pH depending on the p*K* value of pH-sensitive moieties in the membrane phase.

We report here on pH-induced permeability enhancement at a specific pH using an amphiphilic polypeptide, poly(Leu-Gln($EtNH_2$)), membrane composed of L-leucine (Leu) and N^{ω}-(β-aminoethyl)-L-glutamine) (Gln($EtNH_2$)) moieties. That is, the amphiphilic polypeptide membrane exhibited a maximum solute permeability at around pH 4.5, just below the midpoint pH of helix to coil transition of the membrane. This phenomenon can be explained in terms of the helix to coil transition (swelling of the membrane by decreasing pH) caused by pH-induced ionization of Gln($EtNH_2$) groups and the following hydrophobic association (deswelling of the membrane by further decreasing pH) of L-leucine moieties on the induced flexible coil conformation in the membrane.

Experimental Section

Materials. Poly(L-leucine-*co*-γ-methyl L-glutamate) (Poly(Leu-Glu(OMe))). Poly(Leu-Glu(OMe)) was first obtained by polymerization of *N*-carboxy anhydrides of L-leucine (Leu-NCA) and L-glutamic acid γ-methyl ester (Glu(OMe)-NCA) in dichloromethane solution with triethylamine as an initiator.[26] Leu-NCA and Glu(OMe)-NCA were prepared by the reaction between L-leucine (Ajinomoto Co., Ltd.) or γ-methyl L-glutamate (Ajinomoto Co., Ltd), respectively, and phosgen (Hodogaya Chemicals Co., Ltd.) in tetrahydrofuran solution.[27] Their purities, checked

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Scheme 1

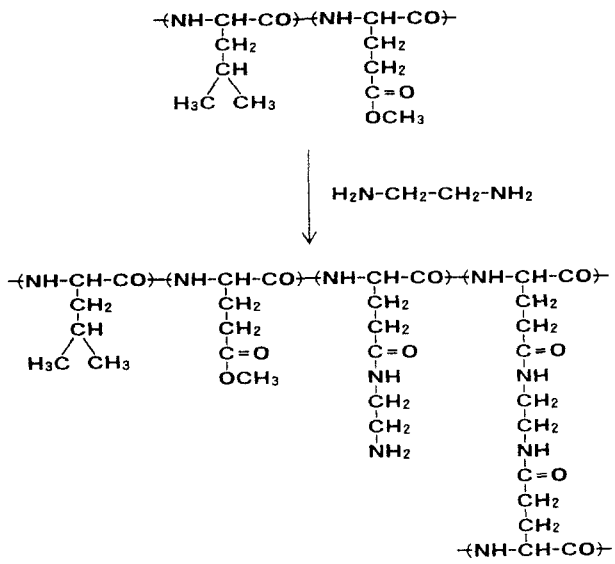


Table 1. Relation between Gln($EtNH_2$) Content and Aminolysis Reaction Time

reaction time/h	30	40	50	60	70	80	90
mol % of Gln($EtNH_2$)	12	18	25	31	34	40	47

by the Mohr method,[28] were 99.9%. The polymerization is as follows: Equimolar quantities of Leu-NCA and Glu(OMe)-NCA were dissolved in dichloromethane which was distilled over calcium carbonate. The solution was stirred and triethylamine was added with stirring. The molar ratio of anhydride to initiator was 200. And then the solution was allowed to stand 24 h at room temperature. The poly(Leu-Glu(OMe)) obtained in 80% yield was precipitated in dry methanol. The intrinsic viscosity of poly(Leu-Glu(OMe)) in dichloroacetic acid was determined by using an Ubbelohde viscometer at 25 °C. A molecular weight of 69 000 was estimated from the intrinsic viscosity by the equation[29] $[\eta] = 2.24 \times 10^{-3} M^{0.58}$. The contents of Leu and Glu(OMe) in the copolypeptide were confirmed to be 49 and 51 mol %, respectively, from ^1H NMR analysis.

The poly(Leu-Glu(OMe)) dissolved in trichloroethylene (ca. 3% solution) was cast on a glass plate, and evaporation was carried out at room temperature. The membrane obtained was washed with methanol for several times and dried in vacuo.

Amphiphilic Polypeptide Consisting of L-Leucine and N^{ω}-(β-(aminoethyl)-L-glutamine) (Poly(Leu-Gln($EtNH_2$))) Membranes. Poly(Leu-Gln($EtNH_2$)) membranes were prepared by aminolysis reaction of poly(Leu-Glu(OMe)) membrane (Scheme 1) in a solvent mixture containing ethylenediamine and 1,2-dichloroethane (10:1 in volume ratio) at 50 °C. The conversion from Glu(OMe) to Gln($EtNH_2$) in the membranes could be monitered by IR spectral changes, e.g., the decrease in absorbance at 1170 cm^{-1} associated with the ester C–O stretching band of Glu(OMe). The contents of Gln($EtNH_2$) residues could be regulated by changing the reaction time. At any reaction time, the overall concentration [Glu(OMe)] + [Gln($EtNH_2$)] in the membrane is constant, 51 mol %, since the Leu residues, 49 mol %, remain unchanged during the reaction. The Gln($EtNH_2$) contents, therefore, were determined by the absorbance at 1170 cm^{-1} of the membrane before (A_b) and after (A_a) the aminolysis reaction, using $(A_b - A_a)/A_b \times 51$ (%), and with the results of elemental analysis (Table 1). It is noted, here, that the Gln($EtNH_2$) content, [Gln($EtNH_2$)], obtained in this manner is as same as the content of Glu(OMe) reacted. In addition, a small amount of Glu(OMe) groups still remain unreacted in poly(Leu-Gln($EtNH_2$)) membranes until the reaction time is increased over 3 days.

The membranes obtained were no longer soluble in organic acids such as dichloroacetic acid and trifluoroacetic acid or in aqueous solution of pH 2–9, indicating that some fraction of ethylenediamine may act as a cross-linking agent during the aminolysis process (Scheme 1). Therefore, [Gln($EtNH_2$)], in Table 1, i.e., the contents of Glu(OMe) reacted include the concentration of N^{ω}-(β-(aminoethyl)-L-glutamine) residues responsible for the formation of cross-link, mol % of Gln(EtNH), which corresponds to cross-linking density of the membranes, ρ. That is, [Gln($EtNH_2$)] is the sum of the net content of Gln($EtNH_2$) and ρ. The net content of Gln($EtNH_2$) groups, [Gln($EtNH_2$)]$_{net}$, on the other hand, was determined by potentiometric counting of the primary amino groups in the membranes. The values of ρ, therefore, could be estimated by subtraction of [Gln($EtNH_2$)]$_{net}$ from [Gln($EtNH_2$)] (Figure 1). Thus, the overall process (Scheme 1) was confirmed to be an amino group introduction/cross-linking reaction between poly(Leu-Glu(OMe)) and ethylenediamine. The thickness of the membrane used in the swelling and permeation experiments was about 35 μm.

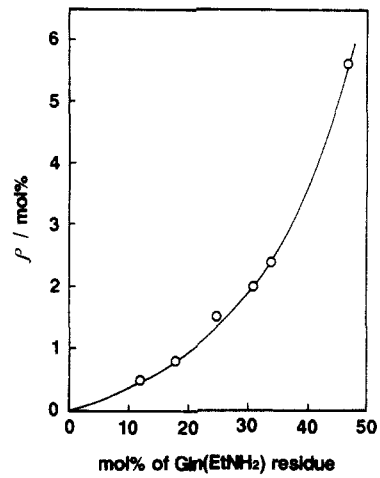


Figure 1. Relation between cross-linking density ρ, mol % of Gln(EtNH), of poly(Leu-Gln($EtNH_2$)) membranes and their Gln($EtNH_2$) content.

Poly(Leu-Gln($EtNH_2$)) Capsule Membrane. The preparation of poly(Leu-Gln($EtNH_2$)) capsule membrane is as follows: Styrene glycol (78 wt %) as a permeation substrate and poly(vinyl alcohol) (Wako Pure Chemical Industries, Co. Ltd., $\overline{DP}$ = 2000, 22 wt %) were mixed together in the presence of a small amount of water and loaded into a hollow cylinder (0.16 cm diameter, 0.30 cm long) of a plastic mold to compress the mixture with a plunger. And then, the mixture was pushed out from the mold and dried in vacuo for 2 days. The mixture dried was immersed into poly(Leu-Glu(OMe)) (10 wt %) dissolved in trichloroethylene and removed from the solution. And then the solvent evaporation was carried out for 24 h at room temperature. Poly(Leu-Glu(OMe)) capsule thus obtained was washed with methanol and dried in vacuo. Finally, poly(Leu-Gln($EtNH_2$)) capsule was obtained by aminolysis reaction of poly(Leu-Glu(OMe)) capsule in a similar manner as above (Scheme 1). The Gln($EtNH_2$) content of poly(Leu-Glu(OMe)) layer of the capsule was 22%. The average thickness of the poly(Leu-Gln($EtNH_2$)) layer was about 20 μm.

The chemicals such as amines, dichloroacetic acid, and the other organic solvents used were of reagent grade. Styrene glycol used was of extra pure grade.

All of the aqueous solutions used contained NaCl (0.1 mol·dm^{-3}) to avoid large molarity changes of the solutions following alternations in pH. HCl and NaOH were used to control the pH of the solutions. The pH values of the solution at around neutral were kept constant during the measurements by titrating with NaOH.

Methods. Degree of Hydration of Membranes. The degree of hydration of the membranes, H, was determined as a weight fraction of water in the water-swollen membrane at 25 °C. The membranes were allowed to swell in an aqueous solution of a prescribed pH. The water-swollen membranes were blotted, weighed, and immersed into the aqueous solution again. This procedure was repeated several times during 24 h by considering the complete conformational transition of the membranes in addition to water sorption equilibrium. No change in weight of the membrane with 35 μm thickness was confirmed to be obtained within 2 h. And then the membranes were dried under reduced

pressure. The H value was calculated from the difference of the weights.

Solute Permeation across the Membrane. Measurements of the permeation of styrene glycol through a membrane were carried out by using a Pyrex glass permeation cell (Asahi Rika Co., Ltd.) composed of two compartments of equal volume (150 mL) at 25 °C. A membrane was clamped between these compartments. Both sides of the cell were filled with an aqueous solution of a prescribed pH and kept for 24 h which is long enough to reach equilibrium of the membranes as mentioned above. After removal of the solution, an aqueous solution of the same pH containing styrene glycol (1.0 mol·dm^{-3}) was introduced into one side (feed side) of the cell and a solute free solution of the same pH was introduced into the other side (permeation side) of the cell. Both of the solutions were stirred slowly to prevent concentration polarization.[30] Solutions of the permeation side were taken out after given periods of time and then ultraviolet absorbance at 256 nm associated with the solute was measured to determine the solute flux, J_s (mol·cm^{-2}·s^{-1}), through the membrane. The permeation coefficient, P_s (cm^2·s^{-1}), was obtained from J_s in the steady state using $P_s = J_s \Delta x/\Delta c$, where Δx is thickness of the swollen membrane and Δc is concentration difference of the solute across the membrane. Poly(Leu·Gln-($EtNH_2$)) membrane containing 31% Gln($EtNH_2$) residues was used.

Solute Release from Capsule Membrane. The capsule coated with poly(Leu·Gln($EtNH_2$)) membrane (20 μm) with 22% of Gln($EtNH_2$) residues was immersed into water (4 mL) of a prescribed pH. The solution was stirred slowly at 25 °C. The release characteristics of styrene glycol from the capsule was followed by detecting increases in the ultraviolet absorbance at 256 nm in the outer water phase. After a time, no change in absorbance was observed. This value of the absorbance is regarded as 100% released. The solute release was also observed replacing the releasing medium pH. In this second method, the membrane was transferred each time to solute-free solutions of different pH, until no change in absorbance increment was observed. The total amount of absorbance increments at respective pH is regarded as 100% released.

Because of the very small volume of the capsule, ca. 3.6×10^{-3} mL, the concentration of styrene glycol in solution inside the capsule was higher than that of its saturated solution (2.1 mol·dm^{-3}) when the percent released was over 90 in both methods. At this time, the concentration of the solute out of the capsule was not over 1.7×10^{-2} mol·dm^{-3} in the former method performed at fixed pH values. Therefore, the concentration gradient of the solute was considered to be almost constant within 90% released. Similarly, the decrease in the solute concentration gradient during the releasing at respective pH was negligibly small in the latter method.

Partition of Solute in Membrane. About 100 mg of poly-(Leu·Gln($EtNH_2$)) membrane containing 31% Gln($EtNH_2$) residues was immersed in an aqueous solution (500 mL) of a prescribed pH containing styrene glycol (0.5 mol·dm^{-3}), and after 10 h the membrane was removed, rinsed, blotted, and immersed in water (15 mL) of the same pH. The amount of the solute extracted from the membrane was obtained in a similar manner to the solute release measurement, neglecting the amount of solute remaining in the membrane after the extracting process. The amount of the solute obtained was the same as that extracted from the membrane immersed again in the same solution for 24 h, thus confirming the partition equilibrium. It could be assumed that the concentration of the solute in the original soaking solution was almost independent of the partition process based on the large difference in volume between the solution (500 mL) and swollen membrane (ca. 0.15 mL). The partition coefficient, K_s, was obtained by $K_s = \bar{c}/c$, where $\bar{c}$ is the moles of the solute per unit volume of swollen membrane at partition equilibrium and c is solute concentration of the original solution.

Spectroscopy. Infrared (IR) and absorption spectra were measured with a diffracting grating infrared spectrometer (Jasco, IR-2) and spectrophotometer (Jasco, UVIDEC 670), respectively. Fluorescence spectra of N-phenyl-1-naphthylamine (NPN), a hydrophobic probe, entrapped in poly(Leu·Gln($EtNH_2$)) membrane containing 31% Gln($EtNH_2$) residues in an aqueous solution were obtained with a spectrofluorophotometer (Shimadzu, RF-540). The membrane was immersed into trichloroethylene (50 mL) containing 10^{-5} mol·dm^{-3} NPN and kept for 2 days. Then, the membrane was removed, rinsed with methanol, and dried in vacuo. The excitation wavelength of NPN was 348 nm. NPN used was of extra pure grade.

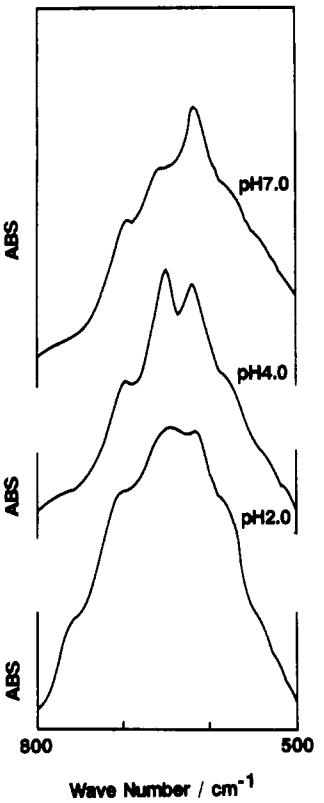


Figure 2. IR spectra of poly(Leu·Gln($EtNH_2$)) membrane with 31% Gln($EtNH_2$) residues at pH 2.0, 4.0, and 7.0.

Potentiometry. Potentiometric measurements of poly-(Leu·Gln($EtNH_2$)) membranes were carried out with a potentiometric automatic titrator (Kyoto electronics, AT-200). The membranes were immersed into an aqueous solution of NaCl (0.1 mol·dm^{-3}). The solution was titrated with an aqueous solution of HCl (1.0 mol·dm^{-3}) in an atmosphere of nitrogen.

Results and Discussion

Conformation of Poly(Leu·Gln($EtNH_2$)) Membranes. In order to examine the conformation of the poly-(Leu·Gln($EtNH_2$)) membranes, IR spectra were measured. In the dry state, poly(Leu·Gln($EtNH_2$)) membranes showed the amide I band at 1650 cm^{-1}, amide II band at 1540 cm^{-1}, and amide V band at 620 cm^{-1}; these are characteristic of α-helical conformation of polypeptides.[31] The membranes with more than 30% Gln($EtNH_2$) groups exhibited a shoulder at 650 cm^{-1} in the amide V band, associated with random coil conformation; however, its intensity was very weak, thus confirming a stable α-helix conformation of poly(Leu·Gln($EtNH_2$)) in the membranes. Furthermore, pH dependence of IR spectra of the water containing membrane with 31% Gln($EtNH_2$) residues is shown in Figure 2. Owing to the strong absorbance of water in the membrane, reliable data of the spectra associated with the membrane matrix were limited to the amide V band region. At neutral pH, the IR spectra indicate the presence of an appreciable amount of α-helix. On the other hand, at pH 2 and 4 a large increase of the band at 650 cm^{-1} suggests loss of α-helix structure and formation of random coil structure. Figure 3 shows the pH dependence of the relative absorbance at 620 cm^{-1} at various pH values to that at pH 7. The membrane, α-helical structured in neutral pHs, undergoes a conformational transition to random coil, when pH is decreased

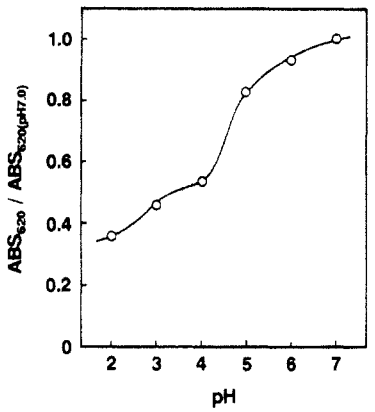


Figure 3. pH dependence of the relative absorbance at 620 cm^{-1} at various pHs to that at pH 7.0.

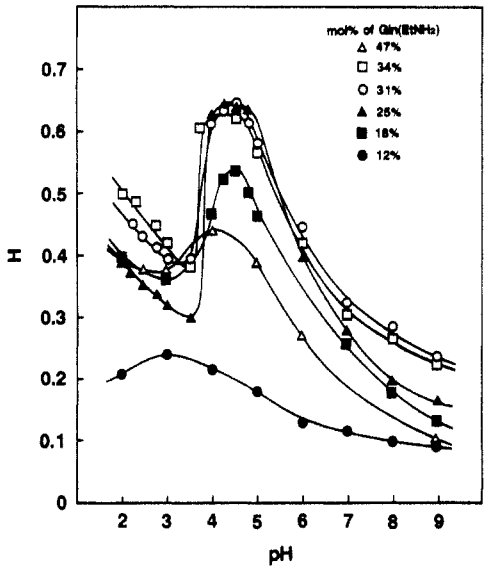


Figure 4. pH dependence of the degree of hydration, H, of poly(Leu-Gln($EtNH_2$)) membranes with various Gln($EtNH_2$) content at 25 °C.

to acid values. The decrease in stability of the α-helix structure in acid pHs can be attributed to the increase in the electrostatic repulsion force between ionizable Gln($EtNH_2$) side chains by decreasing pH. The midpoint for the pH-induced conformational transition is observed at about pH 4.7.

Degree of Hydration of Poly(Leu-Gln($EtNH_2$)) Membranes. Figure 4 shows the water swelling characteristics of poly(Leu-Gln($EtNH_2$)) membranes containing various Gln($EtNH_2$) contents. At around neutral pHs, the degree of swelling (hydration) of the membranes, H, increased with increasing Gln($EtNH_2$) content up to 31%. However, a decrease in H value was observed with the membranes containing more than 34% Gln($EtNH_2$) residues. The H value of the membranes at neutral pHs may be mainly determined by two factors, i.e., side-chain hydrophility and cross-linking density of the membranes. The introduction of hydrophilic Gln($EtNH_2$) residues into the membranes increases both factors (Table 1 and Figure 1) which vary the degree of hydration of the membrane in opposite manners. This is the reason why the membrane containing 31% Gln($EtNH_2$) residue slowed the largest H value at neutral pHs. More remarkable is the pH dependence of the membrane swelling, i.e., each membrane exhibits a maximum value of the degree of swelling at a specific pH depending on the Gln($EtNH_2$) content. It is recognized that the conformation of charged polypeptides is dependent on both pH and molarity of the solution. As

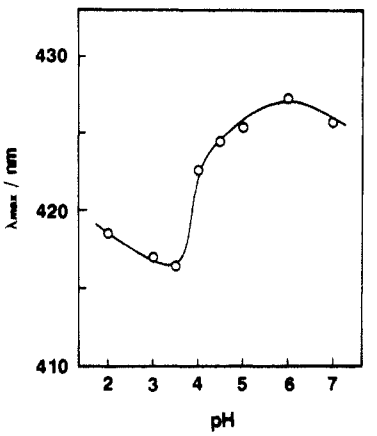
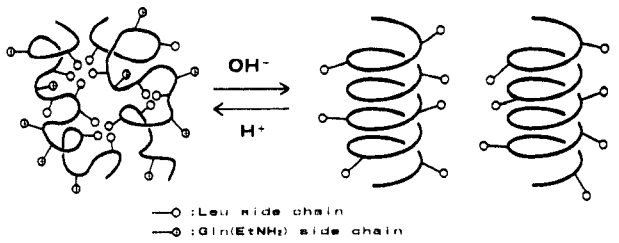


Figure 5. pH dependence of fluorescent emission maxima, λ_{max}, of N-phenyl-1-naphthylamine entrapped in poly(Leu-Gln($EtNH_2$)) membrane with 31% Gln($EtNH_2$) residues.

mentioned previously, the aqueous phase contains 0.1 mol·dm^{-3} NaCl. Therefore, the molarity is considered to be not appreciably changed in the pH range where the swelling–deswelling of the membrane was induced. That is, the main effect in this pH range is the effect of pH rather than molarity. The increase in H value with decreasing pH from 9 to weak acid values, therefore, can be attributed to the increase in the content of charged-coil conformation owing to the partially ionized Gln($EtNH_2$) groups by decreasing pH. It is strange, however, that the membranes were remarkably deswelled by further decreasing pH. Sisido et al.[32] have reported that amphiphilic polypeptides were found to form hydrophobic clusters in aqueous solution even under the charged-coil conformation, which binds fluorescence probes as hydrophobic substrates very efficiently. Figure 5 shows the pH dependence of fluorescent emission maxima, λ_{max}, of N-phenyl-1-naphthylamine (NPN) entrapped in the poly(Leu-Gln($EtNH_2$)) membrane containing 31% Gln($EtNH_2$) residues. It is well-known that fluorescence characteristic of NPN derivatives is its sensitivity to environmental polarity around the probes, e.g., λ_{max} of the probes is shifted to a lower wavelength with decreasing environmental polarity.[33] Above pH 5, λ_{max} of NPN was found to be at around 425 nm, however, the decrease in pH from 5 to 3.5 lowered it to 416 nm, and then slightly higher wavelength shift was observed by further decreasing pH. The hydrophobic NPN should be located in the vicinity of Leu residues in the membrane. Above pH 5.0 the hydrophobic Leu and hydrophilic Gln($EtNH_2$) residues are statistically mixed within the α-helical framework of the polypeptide, so that an association of Leu residues, in which NPN can efficiently be bound, can hardly be made even in the presence of water. This may be reflected in the longer wavelength of λ_{max}. On the other hand, below the midpoint pH, 4.7, of the conformational transition (Figure 3) the random coil conformation is predominant. A steep lowering of the λ_{max} by decreasing pH from 4.5 to 3.5, therefore, implies a formation of hydrophobic clusters between Leu residues under the flexible charged-coil conformation in the water-swollen membrane. Therefore, the remarkable decrease in the degree of swelling of the membranes at around pH 4 (Figure 4) may be ascribed to the fact that the hydrophobic association between Leu residues can be induced under the charged-coil conformation, providing the additional noncovalent cross-linking in the membrane (Scheme 2). On the other hand, the increase in the values of H below pH 3.5 (Figure 4) implies the destruction of Leu clusters. This was consistent with the longer wavelength shift of λ_{max} of NPN below pH 3.5

Scheme 2



(Figure 5). The potentiometric titration data showed that the complete ionization of $Gln(EtNH_2)$ groups in the membranes could be attained at extremely low pH around 2.0. This significant depression of the ionization may be interpreted as pK_a shifts induced by hydrophobicity[15] (Leu or Glu(OMe) residues) in the membranes. Therefore, the destruction of Leu clusters is ascribed to the more increased electrostatic repulsion force between $Gln(EtNH_2)$ residues below pH 3.5. It is also noted, here, that because of a small amount of $Gln(EtNH_2)$, the membrane containing 12% $Gln(EtNH_2)$ residues showed somewhat different swelling characteristics (Figure 4). The ionization degree of $Gln(EtNH_2)$ residue in the membranes would be affected by hydrophobic Leu or Glu(OMe) residues as described above. The neighboring hydrophobic residues effectively depress the ionization degree of $Gln(EtNH_2)$ and keep $Gln(EtNH_2)$ residues apart. In the α-helix conformation the close neighboring positions of $Gln(EtNH_2)$ at the jth unit are $(j \pm 3)$ or $(j \pm 4)$ units. The probability of Leu or Glu(OMe) being located at these positions in the 12% $Gln(EtNH_2)$ content could be estimated to be 62%. Those in other membranes with more than 18% $Gln(EtNH_2)$ decrease below 46%. This difference in the hydrophobicity around $Gln(EtNH_2)$ residue may cause the different swelling character of the membrane with 12% $Gln(EtNH_2)$ residues. Further details are not clear at present.

Solute Permeabilities across the Poly(Leu·Gln-($EtNH_2$)) Membrane. Figure 6a shows the pH dependence of the permeation coefficient, P_s, of styrene glycol across poly(Leu·$Gln(EtNH_2)$) membrane containing 31% $Gln(EtNH_2)$ residues. The shape of the P_s–pH profile is found to be well consistent with the pH-induced swelling/deswelling behavior of the membrane (Figure 4); i.e., the solute permeability could be enhanced at around pH 4.5. In this case the value of P_s at pH 4.5 was 7.5 and 2.5 times as high as that at pH 7.0 and 3.5, respectively.

It has been recognized that the solute permeability across the polymer membrane is determined by two factors: solute partition in the membrane and solute diffusion through the membrane, as shown in eq 1

$$P_s = K_s D_s \qquad (1)$$

where K_s and D_s are partition and diffusion coefficient, respectively. The pH dependence of the diffusion coefficient, D_s, therefore, was obtained from eq 1 with the values of permeability coefficients and K_s values experimentally determined at respective pH (Figure 6a). The result is illustrated in Figure 6b. It is confirmed that the pH dependence of D_s is also quite similar to that of P_s. Therefore, it can be seen from Figures 4 and 6 that the pH dependence of the solute diffusibility and/or permeability is directly related to the specific swelling behavior of the amphiphilic polypeptide membrane.

Table 2 shows the relative values of P_s, K_s, and D_s at pH 4.5 to those at pH 7.0, respectively. It indicates more clearly that a main factor to produce the permeability enhancement, $P_{s(4.5)}/P_{s(7.0)} = 7.5$, is the increase in the

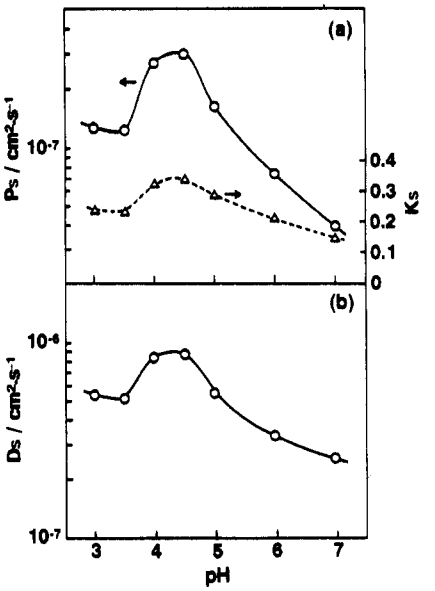


Figure 6. (a) pH dependence of permeation coefficient, P_s, and partition coefficient, K_s, of styrene glycol–poly(Leu·$Gln(EtNH_2)$) membrane with 31% $Gln(EtNH_2)$ system at 25 °C. (b) pH dependence of diffusion coefficient, D_s, of styrene glycol across poly(Leu·$Gln(EtNH_2)$) membrane with 31% $Gln(EtNH_2)$ residues at 25 °C.

Table 2. Relative Values of Permeation, Partition, and Diffusion Coefficients at pH 4.5 to Those at pH 7.0

$P_{s(4.5)}/P_{s(7.0)}$	$K_{s(4.5)}/K_{s(7.0)}$	$D_{s(4.5)}/D_{s(7.0)}$
7.5	2.2	3.4

diffusion coefficient, $D_{s(4.5)}/D_{s(7.0)} = 3.4$, whereas the pH induced variation of the solute solubility is a secondary one. It has been shown, on the other hand, that the diffusion coefficient of water soluble nonionic permeants in polymer membranes effectively reflects the variation of the degree of hydration, i.e., free volume fraction, of the membrane.[34] It may say, therefore, that the changes in the degree of water-swelling of the membrane based on the α-helix to random coil transition (Figure 3) and hydrophobic Leu cluster formation (Figure 5) of poly(Leu·$Gln(EtNH_2)$) in the membrane effectively regulate the solute diffusibility, which results in the solute permeability changes.

Solute Release from Poly(Leu·Gln($EtNH_2$)) Capsule Membrane. The effect of pH on the styrene glycol release from the capsule membrane of poly(Leu·Gln-($EtNH_2$)) with 22% $Gln(EtNH_2)$ residues was also investigated, and the results are shown in Figure 7. It is confirmed, here, from Figure 4 that poly(Leu·$Gln(EtNH_2)$) containing around 20% $Gln(EtNH_2)$ residues also exhibited the specific pH dependence of water-swelling behavior. Similar to the case of the membrane system (Figure 6a), the release rate of styrene glycol exhibited a significant increase at pH 4.5 and at pH values above and below 4.5 the solute release was found to be depressed. The next question was whether this situation was reversible. The results are shown in Figure 8. When the capsule membrane was removed from an aqueous solution of pH 7.0 and placed in an aqueous solution of pH 4.5, a significant increase in solute release immediately took place. When the capsule membrane was next removed and placed in a solution of pH 3.5, a release rate decrease took place. Furthermore, when the capsule membrane was removed from that solution and again placed in a solution of pH 4.5, a significant release rate increase took place, thus confirming a reversibility of the change.

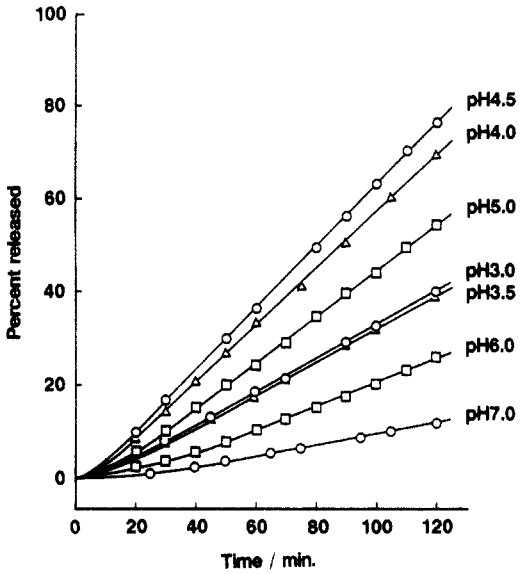


Figure 7. Percent released vs time of styrene glycol from capsule membrane of poly(Leu·Gln($EtNH_2$)) with 22% Gln($EtNH_2$) residues at various pHs at 25 °C.

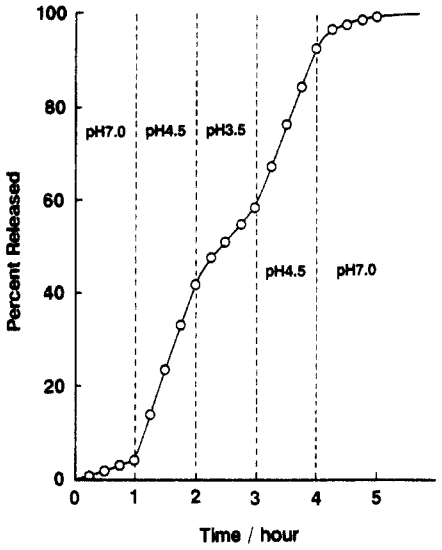


Figure 8. Effect of reversible changes in pH on percentage of styrene glycol released from capsule membrane of poly(Leu·Gln($EtNH_2$)) with 22% Gln($EtNH_2$) residues at various pHs at 25 °C.

In conclusion, the maximum degree of swelling and solute permeability of poly(Leu·Gln($EtNH_2$)) membrane with 31% Gln($EtNH_2$) residues could be obtained at a specific pH region at around 4.5, resulting from the α-helix to random coil transition and the induced hydrophobic leucine cluster formation of the amphiphilic poly(Leu·Gln($EtNH_2$)) in the membrane. The poly(Leu·Gln($EtNH_2$)) was also found to be applicable to the capsule membrane system to control the solute delivery by environmental pH.

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