Control of Solute Permeability Based on pH-Induced Reversible Conformational Change in Block Copolypeptide Membrane

NORIHIKO MINOURA, SEI-ICHI AIBA, and YUKIHIKO FUJIWARA,
Industrial Products Research Institute, Yatabe-machi Higashi, Tsukuba-gun, Ibaraki 305, Japan

Synopsis

An ABBA-type block copolypeptide membrane composed of L-glutamic acid (A) and L-leucine (B) was prepared, and a solute permeability of the membrane was studied. According to the observation of electron microscope, the membrane had the phase-separated morphology that the domains consisting of poly(L-glutamic acid) blocks are embedded in a continuous matrix of the poly(L-leucine) phase. The reversible conformational change of the poly(L-glutamic acid) from α-helix to random coil induced by changing the pH of the external medium was presumed to occur in the domains on the basis of the results of infrared absorption spectra. In the pH dependence of the diffusion coefficients of KCl, NaCl, and LiCl in the membrane, a considerable decrease was found at about pH 4, and thought to result from the conformational transition. In an acidic medium, the permeability of KCl, NaCl, and LiCl was higher than that of glucose, but this order was reversed in higher pHs. These results indicate that the poly(L-glutamic acid) domains in the membrane function as channels for solute transport.

INTRODUCTION

The mechanisms that control membrane permeability are most important in the function of biological membranes. Large domains of proteins existing in the biological lipid bilayer membrane are known to control the permeability of chemical substances such as H^+, Na^+, K^+, Ca^{2+}, amino acids, and sugars. Reversible conformational change of the protein molecule is caused by external stimuli, channels for transport of one specific substrate across the membrane are created, and the specific substrate is allowed to permeate through them.[1]

It was reported that synthetic polymer membranes, for example, porous membranes which anchored poly(L-glutamic acid) in the pore,[2] crosslinked poly(L-glutamic acid) membrane,[3] and butyl methacrylate–L-aspartic acid graft copolymer membrane,[4] could control the permeability of solutes by reversible conformational changes. In these studies, the transport properties of the membranes changed by variation of the pH in the medium.

Many block copolymers display microphase separation and microdomains can be identified in their solid state structure.[5] Capitalizing on this morphological characteristic, it is possible in some cases to produce a membrane having a morphology composed of cylindrical domains in the matrix. The cylindrical domains may extend from one surface of the membrane to the other. In this case, the domains may be regarded as channels for membrane transport.

Journal of Applied Polymer Science, Vol. 31, 1935–1942 (1986)
 CCC 0021-8995/86/061935-08$04.00

This report pertains to a model of the controlled permeability in biological membranes. The purpose of this study is the preparation of a block copolypeptide membrane composed of L-glutamic acid and L-leucine in which the domains act as the part of a transmembrane channel to control the membrane permeability based on pH-induced reversible conformational change of poly(L-glutamic acid) blocks.

EXPERIMENTAL

Materials

An ABBA-type block copolypeptide, (γ-benzyl-L-glutamate)$_x$-(L-leucine)$_y$-(L-leucine)$_y$-(γ-benzyl-L-glutamate)$_x$ (where $x = 0.18, y = 0.32$ mole fraction) was synthesized by a similar procedure to that reported previously.[6] 1,6-Hexamethylenediamine was used as initiator. The mole fractions of γ-benzyl-L-glutamate and L-leucine in the block copolypeptide were estimated from an NMR spectra. The block copolypeptide membrane (0.020 mm thick) was prepared by casting a benzene solution of this polymer on a glass plate to form a film. The block copolypeptide membrane was subsequently hydrolyzed in a mixed solvent consisting of methanol–2-propanol–5*M* NaOH aqueous solution (2:2:1, by volume) for 20 days at 18°C to yield an (L-glutamic acid)$_x$-(L-leucine)$_y$-(L-leucine)$_y$-(L-glutamic acid)$_x$ block copolypeptide membrane. The hydrolyzed membrane was thoroughly washed with methanol, and then dried in air.

Hydration Measurements

The degree of hydration of the membrane; i.e., the volume fraction of water in the water-swollen membrane was determined as follows: The membrane was previously treated with desired pH. Then it was swollen, blotted, and weighed until the constant weight of the swollen membrane was obtained within the experimental error at 20°C. The membrane was then dried to a constant weight under vacuum at 80°C.

Permeation Measurements

Permeation experiments were carried out at 37°C in a plexiglass cell composed of two compartments of equal volume (270 mL). A membrane was clamped in place between these compartments. The solutions of both compartments were adjusted to have the same pH by use of HCl, KOH, NaOH, or LiOH, and salts (KCl, NaCl, LiCl) or glucose were added to one of the compartments to have an initial concentration c_s' of 1.0*M*. The solutions on both sides of the membrane were well stirred. The concentration c_s'' of salts and glucose transported to the other compartment was measured by atomic absorption photometry and high pressure liquid chromatography, respectively. The flux of solutes J_s (mol/cm^2 s) was obtained, and the permeability coefficient P_s (cm^2/s) was calculated by using the following equation:

$$P_s = J_s \cdot L/(c_s' - c_s'') \tag{1}$$

where L is the membrane thickness.

Solubility of Salt in Membrane

A membrane was immersed in a 1.0*M* salt solution of desired pH, and after sorption equilibrium was attained, the membrane was removed, rinsed, blotted, and immersed in distilled water. The amount of the salt desorbed from the membrane to the water was measured by atomic absorption photometry. The partition coefficient K_s was calculated by using the following equation:

$$K_s = \frac{\bar{c} \text{ (mol/L is membrane)}}{c \text{ (mol/L in initial solution)}} \quad (2)$$

Membrane Potential Measurements

Using the plexiglass cell for the permeation measurements, the potentials of the membranes were measured as a function of salt concentration c_s'' at different pH values for the following electrolyte systems: KCl–HCl and KCl–KOH. In these experiments the concentration c_s' of the solution in the compartment (′) was maintained constant and the pH of the solutions in both compartments was equal and kept constant, too. Calomel electrodes can be used as reversible electrodes in all systems. Using the electrodes possessing a salt bridge, we measured the membrane potential directly by means of an electrometer (Model TR-8651, Takeda Riken Co., Japan).

RESULTS AND DISCUSSION

The hydrolyzed block copolypeptide membrane was found to have the microdomain structure as shown in Figure 1. The structure shown may be cylindrical, the black stripes correspond to the domains consisting of poly(L-glutamic acid) blocks embedded in a continuous matrix of the poly(L-leucine) phase. The domains were expected to function as the membrane channels.

The infrared absorption spectra of the hydrolyzed membrane are shown in Figure 2. Characteristic absorptions of the α-helix[7,8] appear at 1650,

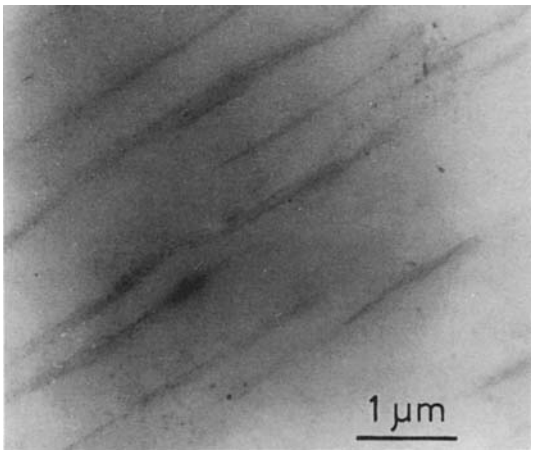

Fig. 1. Electron micrograph of ultrathin section cut vertically to the surface of leucine–glutamic acid block copolypeptide membrane stained by osmium tetroxide.

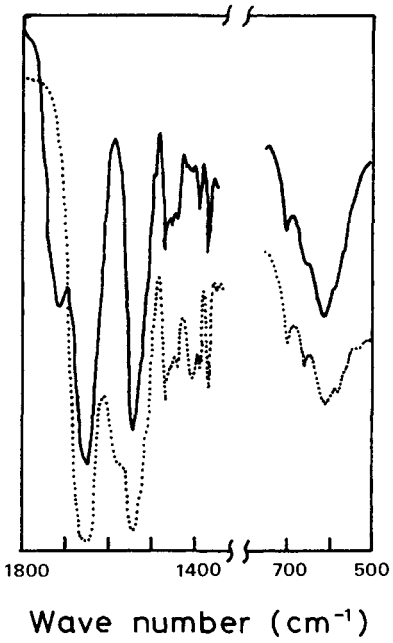

Fig. 2. Infrared absorption spectra of leucine–glutamic acid block copolypeptide membrane; (—) after immersion in an aqueous solution of HCl (pH 2); (- - - -) after immersion in an aqueous solution of NaOH (pH 10).

1545, and 615 cm^{-1} following the immersion of the hydrolyzed membrane in an aqueous solution of HCl (pH 2). After immersion in an aqueous solution of NaOH (pH 10), new absorptions, characteristic of the random coil,[7,8] appear at 650 and 1580 cm^{-1} in addition to the absorption bands of the α-helix. Such spectral changes caused by the pH alteration were reversible. This indicates that a reversible conformational change from α-helix to random coil is indeed induced by the pH alteration and is presumed to occur in the domains consisting of the poly(L-glutamic acid) block in the hydrolyzed membrane.

In order to get sufficient information regarding fixed charges (COOH groups) in the hydrolyzed membranes, the membrane potential was studied as a function of salt concentration. The effective fixed charge concentration in the membranes will be affected by the pH of the external solution because the membranes possess the carboxyl groups. Figure 3 shows the membrane potentials as a function of KCl concentration c_s'' at different pH values. The curves of the measured membrane potential coincide with those of the theoretically established membrane potential at different pH values, taking into account a dissociation equilibrium of the carboxyl group.[9] Therefore, the effective fixed charge concentration C_x (eq/L) in the membrane is obtained from the measured membrane potential curve (Fig. 3) using the following relationship[9]:

$$C_x = 2 \cdot z \cdot K_s \cdot c''_{s-\max} \cdot \frac{U^2}{1 - U^2} \tag{3}$$

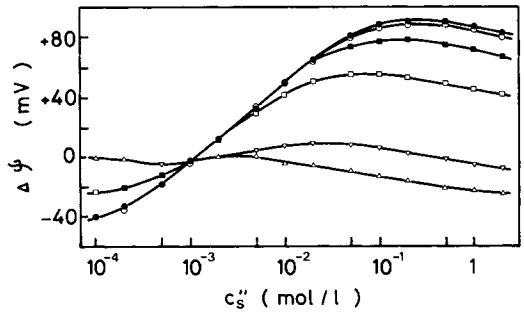

Fig. 3. Membrane potential $\Delta\Psi$ as a function of KCl concentration c_s'' = 0.001M KCl): (△) pH 2; (▽) pH 3; (□) pH 4; (■) pH 5.4; (○) pH 7; (●) pH 8.5. T = 298 K.

where z is valency, K_s is salt partition coefficient, $c''_{s-\max}$ is the value of the salt concentration where $\Delta\Psi$ reaches maximum (as shown in Fig. 3), and U is the transport number obtained from the slope of the straight line at the higher salt concentration region of the membrane potential curves (Fig. 3).

Figure 4 shows the values of the effective fixed charge concentration C_x obtained at different pH values of the external solutions. C_x increases significantly over pH 4. This behavior is similar to that observed in the pH dependence of the fraction of the ionized groups in solutions of poly(L-glutamic acid).[10] It is recognized that the carboxyl groups in the poly(L-glutamic acid) domains are ionized in the pH range above 4, and the macromolecules conform to some extent into random coil forms in that pH range.

Many charged membranes are known to display a high degree of hydration and to swell. Figure 5 shows the degree of hydration of the hydrolyzed membrane at different pH values. The pH dependence of the degree of hydration is rather small, though the degree of hydration is proportionately greater with increasing pH in the higher pH ranges. The degree of hydration of the hydrolyzed membrane should be about 0.35 at pH 5.2 if the additivity rule for hydration of the corresponding homopolymers [e.g., poly(L-glutamic acid) and poly(L-leucine)] can be applied. This result suggests that the water sorption on the membrane and the swelling of the membrane are restricted because the hydrophilic domains consisting of poly(L-glutamic acid) blocks are embedded in a continuous matrix of the hydrophobic poly(L-leucine) phase.

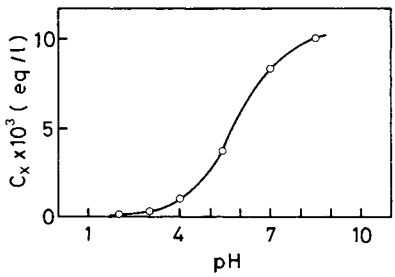

Fig. 4. Effective fixed charge concentration C_x as a function of pH of external solution.

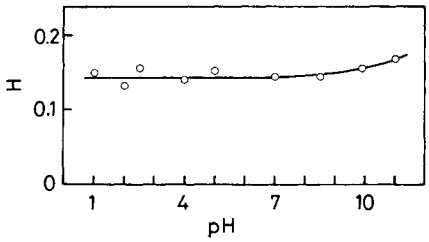

Fig. 5. Degree of hydration H as a function of pH.

Permeation experiments of LiCl, NaCl, KCl, and glucose were carried out at different pH values. the solutes were found to permeate through the hydrolyzed membrane. However, the solutes could not permeate through the block copolypeptide membrane before hydrolysis. This result suggests that the hydrophilic domains, consisting of the poly(L-glutamic acid) block, form continuous phases from one surface to the other across the hydrolyzed membrane. Thus, these continuous phases function as channels for solute transport.

Figure 6 shows that the permeability coefficients of the ionic solutes in the hydrolyzed membrane decrease inversely to increasing pH, while that of the nonionic solute (glucose) increases. The decrease is mainly due to the ionic repulsion between the ionic solutes and carboxylate groups of the poly(L-glutamic acid) in the hydrolyzed membrane, and the increase may be due to better solubility of glucose in the membrane. In an acidic medium, the permeability of KCl, NaCl, and LiCl is higher than that of glucose, and this is reversed in the higher pH ranges. This characteristic was found to be reversible; thus, solute permeation is indeed controlled by changing the pH of the external medium.

Figure 7 shows the pH dependence of the partition coefficients of salts for the hydrolyzed membrane. The partition coefficients reach a constant value at low pHs, and then increase at approximately pH 3–4. After that, the partition coefficients reach a constant value at high pH values, or decrease with increasing pH. The decrease is due to the effect of the Donnan

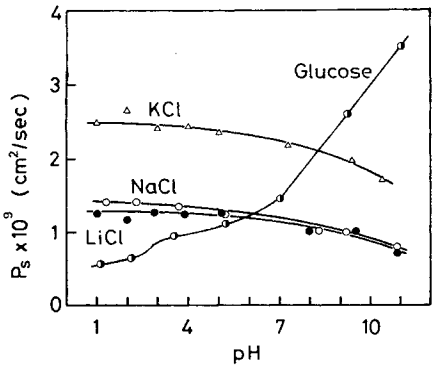

Fig. 6. Permeability coefficient P_s vs. pH of external solution.

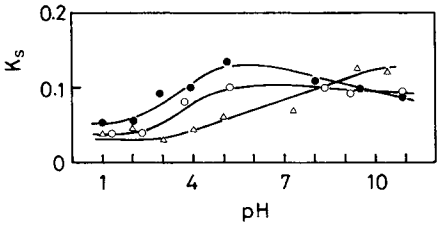

Fig. 7. Partition coefficient K_s vs. pH of external solution. Symbols are the same as shown in Figure 6.

exclusion, because the effective fixed charge concentration increases with pH, as shown in Figure 4.

Diffusion coefficients were obtained by dividing the permeability coefficients by the partition coefficients, and are shown in Figure 8. The diffusion coefficient of salts in the hydrolyzed membrane was found to reach a constant value at low pHs, and then sharply decrease at about pH 3–4. These diffusion characteristics seem to be induced by the transformation from α-helix to random coil form of the poly(L-glutamic acid). The high diffusibity at low pHs is due to the ion diffusion in the side-chain regions between the helices of the poly(L-glutamic acid), where the local molecular density is low and the mobility of the side-chain is high. At high pHs, the low diffusibity is due to the random coil structure of the poly(L-glutamic acid) molecules and the electrostatic interaction between the carboxylate groups and penetrant ions. We have elucidated that the diffusion of gases and vapors in polypeptides with α-helical structure occurs in the side-chain regions between helices; therefore, the diffusibity is high.[11,12]

The pH where the diffusion coefficients sharply decrease differs dependent upon the variety of salts. Especially in LiCl, a helix–coil transition occurs at about pH 3 as shown in Figure 8. This value seems to be too small. In general, the helix–coil transition of polypeptides is affected by variety and concentration of additional salts, temperature, and so on. The pH at which the conformational transition occurs is known to shift toward a low value with decreasing crystallographic radius of the cation of additional salts, and also with increasing concentration of additional salts.[10,13] In the present study, the solubilities of KCl, NaCl, and LiCl in the membrane

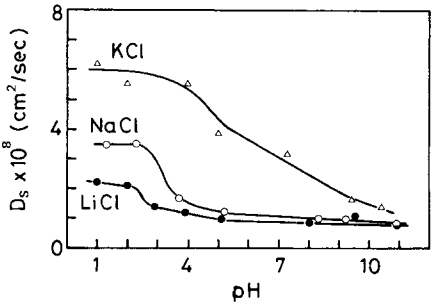

Fig. 8. Diffusion coefficient D_s vs. pH of external solution.

increase in that order in the range of pH 2–5 (Fig. 7). Accordingly, the shift of the transition point toward a low pH value is attributable to both effects of the variety and concentration of the additional salts, and it seems to be reasonable that for the diffusion of KCl, NaCl, and LiCl in the membrane the pH at the conformational transition point decreases in that order.

In conclusion, the block copolypeptide membrane had the phase-separated morphology that the domains consisting of poly(L-glutamic acid) blocks are embedded in a continuous matrix of the poly(L-leucine) phase. In the domains, the reversible conformational change of the poly(L-glutamic acid) from α-helix to random coil induced by changing the pH of the external medium occurred. The solute permeation through the membrane was controlled by the reversible conformational change. Poly(L-glutamic acid) domains in the block copolypeptide membrane simulate the transmembrane channels which are found in biological membranes.

The authors wish to thank Professor T. Iijima of Tokyo Institute of Technology for helpful discussions.

References

1. (a) D. L. Oxender, *Annu. Rev. Biochem.*, **41**, 777 (1972); (b) W. R. Penrose, R. Zand, and D. L. Oxender, *J. Biol. Chem.*, **245**, 1432 (1970); (c) Y. Kagawa, *Biochim. Biophys. Acta.* **505**, 45 (1978).

2. E. Pefferkorn, A. Schmitt, and R. Varoqui, *Biopolymers*, **21**, 1451 (1982).

3. T. Kinoshita, A. Takizawa, and Y. Tsujita, *Nippon Kagaku Kaishi*, **1983**, 868.

4. M. Maeda, M. Kimura, Y. Hareyama, and S. Inoue, *J. Am. Chem. Soc.*, **106**, 250 (1984).

5. G. E. Molau, *Block Polymers*, S. L. Aggarwal, Ed., Plenum, New York, 1970, p. 79.

6. T. Hayashi, A. G. Walton, and J. M. Anderson, *Macromolecules*, **10**, 346 (1977).

7. C. H. Bamford, A. Elliott, and W. E. Hanby, *Synthetic Polypeptides*, Academic, New York, 1956, Chap. 5.

8. T. Miyazawa, *Poly-α-Amino Acids*, G. D. Fasman, Ed., Dekker, New York, 1967, Chap. 2.

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

10. M. Nagasawa and A. Holtzer, *J. Am. Chem. Soc.*, **86**, 538 (1964).

11. N. Minoura and T. Nakagawa, *J. Appl. Polym. Sci.*, **23**, 815 (1979).

12. N. Minoura, *J. Appl. Polym. Sci.*, **27**, 1007 (1982).

13. H. Kanehiro, J. Komiyama, and T. Iijima, *Nippon Kagaku Kaishi*, **1980**, 254.

Received June 3, 1985
Accepted August 26, 1985