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Shoheiinoue[a]; Mizuo Maeda[a]; Dae-Won Chung[a]; Masato Aoyama[a]
[a] Department of Synthetic Chemistry Faculty of Engineering, University of Tokyo Hongo, Bunkyo-ku, Tokyo, Japan

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BIOMEMBRANE MODEL FROM STABLE POLYMER MEMBRANE HAVING POLYPEPTIDE DOMAINS AS CHANNELS

SHOHEI INOUE,* MIZUO MAEDA, DAE-WON CHUNG, and MASATO AOYAMA

Department of Synthetic Chemistry
Faculty of Engineering
University of Tokyo
Hongo, Bunkyo-ku, Tokyo 113, Japan

ABSTRACT

A novel polymer membrane having a transmembrane permeation pathway (channel) was prepared from a polyvinyl-polypeptide graft copolymer. The transmembrane continuous phases of the hydrophilic polypeptide were found to be formed in the stable matrix from vinyl polymer and to function as a permeation pathway for polar substances; the polypeptide domain is regarded as a membrane protein model. The infrared and circular dichroism spectra of the membrane showed the pH-dependent conformational change of the polypeptide segment. Regulation of permeability and permselectivity was performed by pH based on the conformational transition of the channel-composing polypeptide. In addition, the membrane has been found to respond to divalent cations, cationic surfactants, urea, and organic solvents in terms of membrane permeability as well as conformational status of polypeptide.

INTRODUCTION

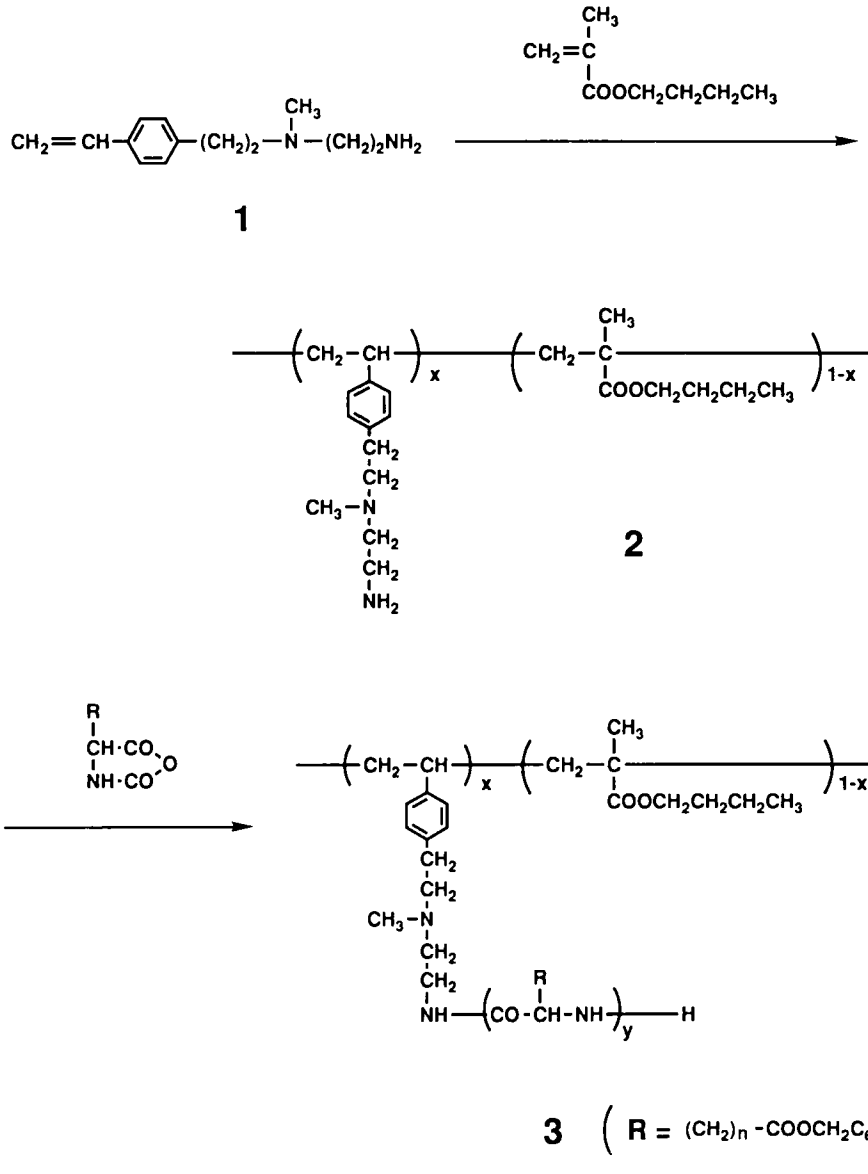
Biomembranes consist of a continuous nonpolar hydrocarbon matrix from a phospholipid bilayer substantially impermeable for most polar substances and protein molecules capable of transporting specific sub-

strates across the membrane even against a concentration gradient. In addition to specific, facilitated, and/or active transports, important and interesting functions of the proteins involve signal transduction. The protein molecules may undergo stimuli-responsive conformational changes to create a "hole" or a "channel" in the membrane for the specific substrate transported.

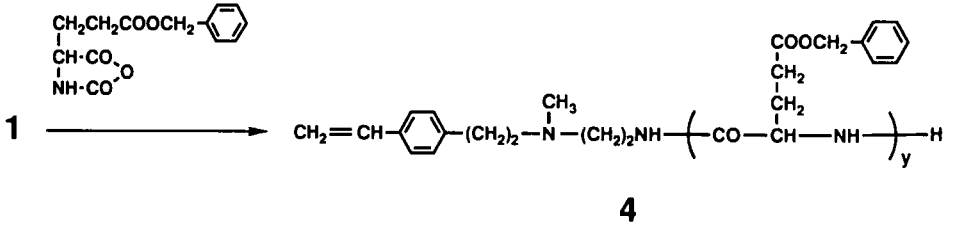
We describe herein the preparation of synthetic membranes that share with their natural counterparts several important characteristics: 1) The synthetic membranes have hydrophilic domains which are distributed like a mosaic in a hydrophobic matrix; 2) the hydrophobic part of the membrane forms a permeation barrier like a lipid bilayer, although it is much more stable, like building blocks; 3) the hydrophilic domains have a polypeptide structure like membrane proteins, serving as permeation pathways ("channel") for polar substances; and 4) the membranes respond to chemical and physical signals via conformational changes of the channel-composing polypeptides in terms of permeability and/or permselectivity.

PREPARATION

The design of membranes with microdomains of a polypeptide in the matrix of stable polymers is based on the synthesis of vinyl polymers with polypeptide branches; polyvinyl-polypeptide graft copolymers [1]. Poly(butyl methacrylate), which is known to have excellent mechanical properties, was adopted as the backbone. The branch was composed of a hydrophilic polypeptide including poly(L-aspartic acid), poly(L-glutamic acid), and poly(*N*-hydroxyalkyl-L-glutamine). The starting material for the preparation of the graft copolymer (**3**) is a styrene derivative having a primary amino group (**1**) [2, 3]. The radical copolymerization of **1** and butyl methacrylate gave the backbone copolymer (**2**). Then **3** was synthesized by the polymerization of *N*-carboxylic anhydride (NCA) of β-benzyl L-aspartate (BLA) or γ-benzyl L-glutamate (BLG) initiated by the primary amino group of **2** (Scheme 1). As for the graft copolymer having poly(*N*-hydroxyalkyl-L-glutamine) branches, a macromonomer method [4] was applied: a polypeptide macromonomer (**4**) was prepared by the polymerization of BLG-NCA initiated with **1**, and was converted to poly(*N*-hydroxyalkyl-L-glutamine) macromonomer (**5**) by reaction with an amino alcohol. **5** was then copolymerized with butyl methacrylate to afford **3** [5] (Scheme 2).



SCHEME 1.



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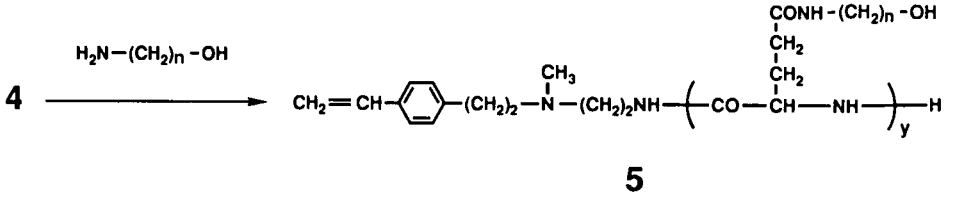

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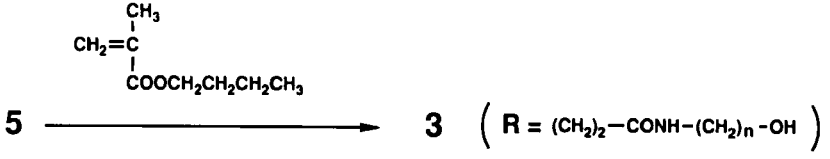

SCHEME 2.

Preparation of membranes was typically carried out as follows: The graft copolymer (**3**; R = $(CH_2)_nCOOC_6H_5$) was cast on a glass plate from a chloroform solution. A uniform and transparent membrane with a thickness of ca. 40 μm was obtained and was fixed vertically in the center of a diaphragm-type cell. The effective area of the membrane was 3 cm^2. The as-cast membrane is impermeable to ions. In order to convert the benzyl ester group of the polypeptide branch to a carboxylate group, the membrane was subjected to alkaline hydrolysis. After the procedure, the membrane was still self-standing and fully transparent. The removal of the benzyl group was followed by infrared (IR) absorption of the monosubstituted phenyl group. The amount of benzyl alcohol released from the membrane was determined by ultraviolet (UV) spectrophotometry [6, 7].

MEMBRANE STRUCTURE AND PERMEABILITY

Graft copolymers with various numbers and lengths of polypeptide branches were synthesized [7]. Composition and permeability of the graft copolymer membranes are summarized in Table 1. It can be seen that the number and the length (degree of polymerization, DP_n) of the polypeptide branch in the graft copolymer have a remarkable effect upon the permeability of sodium ion and the mechanical strength of the membrane after hydrolysis. All the membranes that were not treated with the hydrolyzing solution were impermeable to ions. For Membranes 3, 4, 7, and 8, which were subjected to hydrolysis, the permeation of sodium ion and sufficient strength were observed. Membranes with lower polypeptide contents, i.e., Membranes 2, 6, and 7, were quite impermeable to sodium ion after hydrolysis. On the other hand, mem-

TABLE 1. Composition and Permeability of Graft Copolymer Membranes

	Composition[b]				
No.	n	% branching, x	DP of branch, y	Peptide content,[c] unit mol%	Permeability for Na^+ after hydrolysis[d]
1[a]	—	0	0	0	No
2	1	3.5	13	32	No
3	1	10	8	47	Yes
4	1	4.8	21	50	Yes
5	1	10	21	70	(Too brittle)
6	2	1.2	19	18	No
7	2	1.2	28	25	No
8	2	3.5	10	28	Yes
9	2	3.8	16	39	Yes
10	2	12	7	45	(Dispersed in water)
11	2	12	13	60	(Dispersed in water)

[a]Poly(butyl methacrylate) homopolymer.
[b]See Scheme 1: **3**, R = $(CH_2)_nCOOCH_2C_6H_5$.
[c]Calculated from x and y.
[d]For NaCl in water.

branes with higher polypeptide contents, i.e., Membranes 5, 10, and 11, were rather weak or dispersed in water after hydrolysis. Sodium ion did not permeate through the membrane from a homopolymer of butyl methacrylate (Membrane 1) which was subjected to hydrolyzing conditions. The IR spectrum of the membrane did not change before and after this treatment. Therefore, the hydrolysis of butyl ester in the backbone of the graft copolymers may be excluded under the present conditions of hydrolysis.

Block and graft copolymers are known to have a tendency to form a microphase-separated structure in the solid state because of little or no compatibility among different polymer segments. In fact, some of those containing polypeptide segments were reported to have microdomains composed of polypeptide [8]. This is also the case for the present graft copolymer membranes: microphase-separated structures were observed for ultrathin sections of the graft copolymer membrane (Membrane 4) after hydrolysis by the use of transmission electron microscopy [7]. The formation of transmembrane continuous phases from hydrophilic polypeptide in the stable matrix of vinyl polymer thus accounts for the manifestation of the permeability for polar substances with sufficient strength in Membranes 3, 4, 8, and 9. The morphology of the microdomain structure should depend upon the composition of the copolymer. In membranes with a lower polypeptide content (Membranes 2, 6, and 7), polypeptide segments are considered to form spherelike, discrete domains, so that hydrolysis cannot proceed in the interior of the membrane to form hydrophilic permeation pathways. On the contrary, in membranes having a higher polypeptide content (Membranes 4, 10, and 11), vinyl polymer segments are considered to form discontinuous, spherelike microdomains so that they cannot play a significant role as a supporting matrix.

pH-DEPENDENT PERMEATION AND CONFORMATION

The continuous phases of polypeptide were thus suggested to be formed in the stable polymer membrane and to function as permeation pathways for ions (transmembrane channel). It is known that conformational changes occur in synthetic polypeptides due to their surroundings including pH, ions, chemical substance, solvent composition, etc. We therefore expect that solute permeability can be controlled by conforma-

tional change of the polypeptide segment in the membrane responding to these external stimuli.

Figure 1 shows circular dichroism (CD) spectra of the film of the Copolymer 9 before and after hydrolysis [9]. Before hydrolysis the CD spectrum showed a negative curve centered at 226 nm, which indicates that poly(γ-benzyl-L-glutamate) chains (branch) in the membrane mainly take a right-handed α-helical conformation. When the hydrolyzed film was pretreated with a buffer solution of pH 2, the CD spectrum exhibited a negative curve centered at 223 nm but of lower intensity than that of the film before hydrolysis. The negative peak indicates the formation of a right-handed α-helix, in a manner similar to poly(L-glutamic acid) in acidic aqueous solution. The CD spectrum of the film after pretreatment

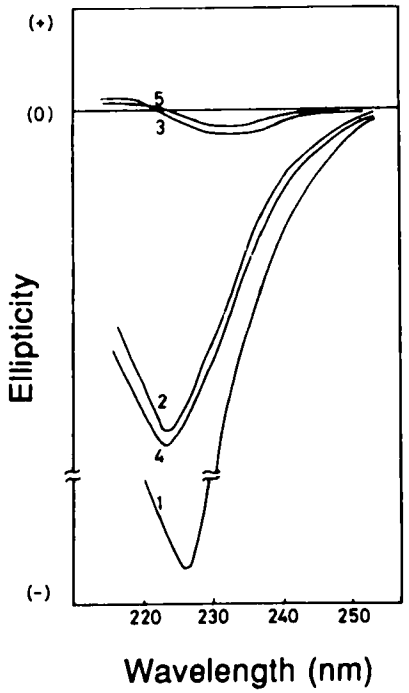

FIG. 1. Circular dichroism spectra of the film from Graft Copolymer 9: 1, before hydrolysis; 2, after hydrolysis, at pH 2; 3, after hydrolysis, at pH 7; 4 after hydrolysis, at pH 2; 5 after hydrolysis, at pH 7. Numbers represent the order of measurement.

with a buffer solution of pH 7 showed a pronounced decrease in the intensity at 223 nm, which indicates the transformation from α-helix to the random coil form. This behavior was reversible; upon lowering the pH from 7 to 2 and increasing it from 2 to 7, almost the same CD curves appeared again at the same pH, respectively, as seen in Fig. 1. When the branch was poly(L-aspartic acid), a similar change in CD spectra with pH was observed [1]. IR spectra have also suggested the conformational transition of poly(L-aspartic acid) branches in Membrane 4 induced by pH changes [7].

Figure 2 shows the permeation rate of styrene glycol across Membrane 4 after hydrolysis in buffer solutions with various pH values [7]. The sigmoidal shape of the rate-pH profile demonstrates the significant change in permeability of the membrane in the region around pH 4.5–5. The pH-dependent permeability change is explained by the structural change of the polypeptide branches as observed in CD and IR spectra: the dissociation of carboxyl groups brings about the conformational change of the pathway ("channel")-composing polypeptide chains between ordered and disordered forms, resulting in changes in hydrophilicity and mobility of the polypeptide domain.

Permselectivity of sugars (glucose, lactose, raffinose) and styrene glycol as mixtures was investigated for the graft copolymer membranes [9]. Permeation rates were in the order styrene glycol > glucose (monosac-

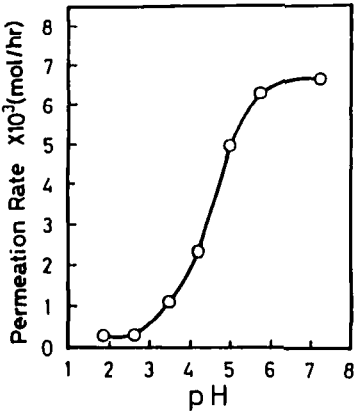

FIG. 2. Rate of permeation of styrene glycol (initial concentration, 0.1 *M*) across Membrane 4 after hydrolysis, in buffer solutions with various pH at 30°C.

charide) > lactose (disaccharide) > raffinose (trisaccaride) in the pH region 1.8–7.3. The relative permeability of sugars to styrene glycol and of lactose and raffinose to glucose was much more diminished in the low pH region than in the high pH region. The permeability of glucose to raffinose, e.g., in Membrane 4, was doubled at pH 7 and ten times higher at pH 2. Permselectivity as well as permeability was thus regulated by a change in pH.

SIGNAL RESPONSIBILITY

Ca^{2+} ion is known to act as a second messenger for information transduction in the life process. The graft copolymer membrane was found to respond to divalent cations such as Ca^{2+} in terms of permeability [6]. The permeability of styrene glycol was remarkably reduced in the presence of divalent ions and was recovered by the addition of ethylenediaminetetraacetic acid (EDTA) as shown in Fig. 3. The IR spectra of the membrane demonstrated a reversible change of the structure of the

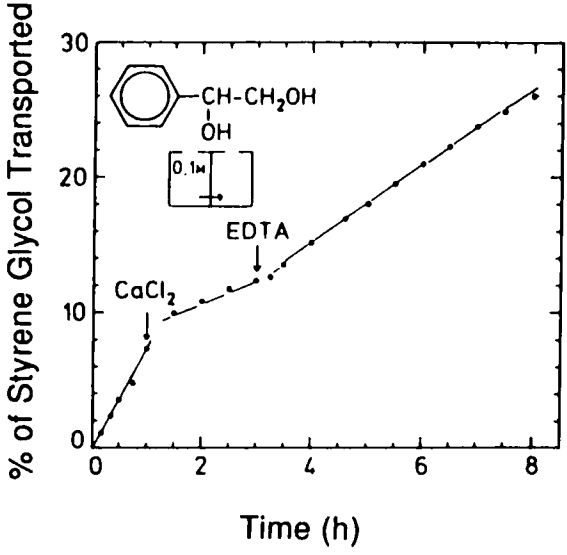

FIG. 3. Reversible permeability change of styrene glycol across the membrane [with poly(L-glutamic acid) branch; degree of branching, 4%; DP_n of branch, 15] using $CaCl_2$(aq) and ethylenediaminetetraacetic acid (EDTA) (aq).

pathway-composing polypeptide chains induced alternatingly by Ca^{2+} and EDTA.

Cationic surfactants are known to induce conformational changes in poly(L-glutamic acid). The effects of three cationic surfactants, dodecylammonium chloride ($C_{12}N^+$), dodecyldimethylammonium chloride ($C_{12}N^+2C$), and dodecyltrimethylammonium chloride ($C_{12}N^+3C$), on the permeability of styrene glycol were investigated for a graft copolymer membrane containing poly(L-glutamic acid) domains as a permeation pathway [10]. When it was treated with a surfactant, the membrane showed a decrease in permeability due to the coil-to-helix transition of poly(L-glutamic acid) induced by the surfactant. The effects of the three surfactants on the permeability were found to be of the order: $C_{12}N^+ > C_{12}N^+2C > C_{12}N^+3C$. The helix content as estimated by CD measurements on membranes treated with the three surfactants was in the order parallel to the effect on permeability.

The effects of cationic surfactants with alkyl groups of different chain

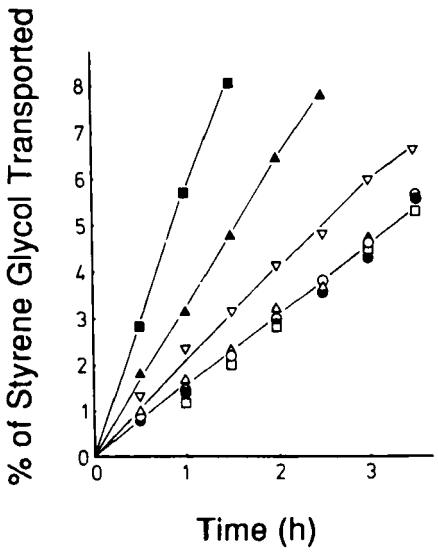

FIG. 4. Time-transport curves of styrene glycol (initial concentration, 0.1 *M*) across Membrane 9 after hydrolysis: after treatment with 0.1 *M* HCl [(1) (○), (3) (●), (5) (△), (7) (□)], with 1 *M* urea [(2) (▽)], with 4 *M* urea [(4) (▲)], with 8 *M* urea [(6) (■)]. Numbers represent the number of the permeation experiments.

lengths on the graft copolymer membrane were also examined [11]. Decyl (C_{10}), dodecyl (C_{12}), tetradecyl (C_{14}), hexadecyl (C_{16}), and octadecyl (C_{18}) trimethylammonium chlorides were investigated on the conformation of poly(L-glutamic acid) chains in the membrane, and on the permeability of styrene glycol across the membrane. For the membrane pretreated with NaOH, C_{10}, C_{12}, and C_{14} induced coil-to-helix transition, but C_{16} and C_{18} did not. For the membrane pretreated with HCl, C_{16} and C_{18} induced helix-to-coil transition, but C_{10}, C_{12}, and C_{14} did not. In most cases, treatment of the membrane with surfactants brought about a decrease in the permeability of styrene glycol, except for the effects of C_{16} and C_{18} which enhanced permeability across the HCl-pretreated membrane.

Thus, the effects of surfactants on permeability depend upon their structures. This means that the membrane may recognize the difference in the structure of signal compounds and respond to them in different ways.

Urea is widely used as a denaturant for proteins. The effect of urea on the helix-coil transition of poly(L-glutamic acid) has been studied; urea is known to favor the random-coil form. Its effect on permeability was investigated for the present membrane [12]. The membrane treated with urea showed an increase in permeability, as seen in Fig. 4. At the same time, the conformational change from α-helix to random coil was observed for poly(L-glutamic acid) chains in the membrane by CD spectral studies (Fig. 5). On the other hand, IR studies showed that urea treatment did not affect the dissociation of the carboxyl groups of poly(L-glutamic acid) chains in the membrane. Interestingly, the membrane treated with urea was found to become less selective to the molecular size of permeating sugars. This behavior may be regarded as a "denaturation" process for the membrane, based on the conformational change of the polypeptide chain.

EFFECT OF CONFORMATION

The permeability of sodium ion, styrene glycol, or sugars was thus able to be regulated for graft copolymer membranes by conformational change of the poly(L-aspartic acid) or poly(L-glutamic acid) chains induced by pH, divalent cations, cationic surfactants, or urea. However, the conformational transition of these polypeptides is, in most cases, accompanied by a change in the extent of ionization of the carboxyl

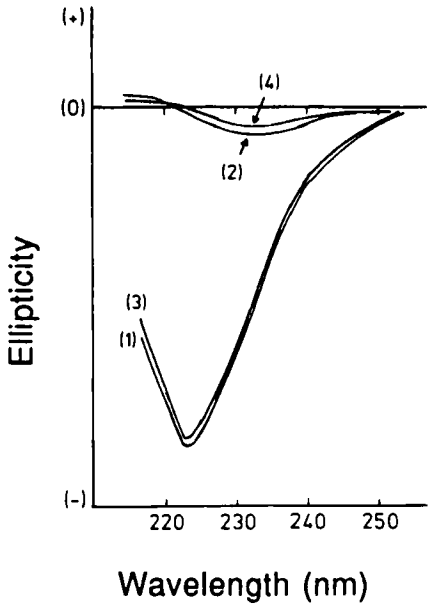

FIG. 5. Circular dichroism spectra of the film from Graft Copolymer 9 after hydrolysis; treated with (1) 0.1 *M* HCl, (2) 8 *M* urea, (3) 0.1 *M* HCl, (4) 0.1 *M* NaOH. Numbers represent the order of measurement.

groups, which should result in a change in hydrophilicity of the polypeptide domains. Therefore, it is difficult to discuss the effects on permeability separately in terms of these two factors.

In order to clarify the effect of the conformational change of the polypeptide branch on permeability, a graft copolymer membrane from butyl methacrylate and the macromonomer **5** was prepared [5]. The polymer has as its branch a non-ionizable, hydrophilic polypeptide, poly(*N*-hydroxyalkyl-L-glutamine), whose conformation has been reported to depend on the hydrophobicity of the solvents. In fact, the membrane showed a CD spectra characteristic of a polypeptide with a right-handed α-helix in a water–methanol mixture (1:1), while it showed the random-coil form in water. The permeability of styrene glycol across the membrane was found to be affected by the solvent: the permeability of the membrane in water/methanol is lower than that in water. Thus, the lower permeability in the water/methanol mixture than in water is considered to be due to the rigid α-helical form of polypeptide chains in the membrane.

CONCLUSION

In biological membranes, conformational transitions in membrane-bound macromolecules are believed to provide the most general and powerful mechanisms for chemical and physical processes. In this context, a synthetic membrane having polypeptide domains can be regarded as a good model of biomembranes, since the mechanisms of permeability regulation involve stimuli-responsive conformational changes of the polypeptide chains which compose the permeation pathway in the membrane. The concept has recently been extended to the photoregulation of membrane permeability; the permeability across a membrane containing a photoresponsive polypeptide was regulated by a photoinduced conformational change of the polypeptide chains [13].

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