pH-Induced Regulation of Permselectivity of Sugars by Polymer Membrane from Polyvinyl-Polypeptide Graft Copolymer

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Abstract: Permselectivity of sugars (glucose, lactose, raffinose) and styrene glycol in their mixture and its pH-induced regulation were investigated for the polymer membrane from poly(butyl methacrylate)-poly(L-glutamic acid) or -poly(L-aspartic acid) graft copolymer. Permeation rates were in the order styrene glycol > glucose > lactose > raffinose in the region of pH 1.8-7.3. Relative permeability of sugars to styrene glycol and of lactose and raffinose to glucose was much more diminished in the region of low pH than in the region of high pH.

Selective transport of sugars across the biomembrane is of importance in life process, where carrier protein plays an essential role. One example of carrier protein in the sugar transport system is the lactose carrier of the cytoplasmic membrane of *Escherichia coli* which consists of 12 α-helical segments with ca. 24 amino acid residues per segment that perpendicularly traverse the membrane.[1] D-Glucose carrier of the membrane of human erythrocyte also transports D-galactose, D-mannose but not L-series corresponding to these sugars.[2] Sugars are transported selectively in intestine by their size,[3] and in dependence on pH.[4] Thus, the transport of sugars across the biomembrane shows the highly regulated permselectivity.

On the other hand, there have been only a few reported studies examining the permselectivity of sugars by artificial membrane, although cellulose acetate membranes, for example, have been used in order to separate oligosaccharides from polysaccharides by ultrafiltration or reverse osmosis in food industry. The permeability of mixed aqueous solutions of protein and sugars (glucose, raffinose) for poly(methacrylic acid) membranes treated and untreated with poly(ethylene glycol) having the "chemical valve" function was investigated.[5] However, no difference in permeation rate of glucose and raffinose was observed.

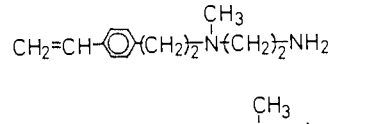
Recently, we have prepared a new type of biomembrane model which is composed of vinyl polymer having a polypeptide branch: poly(butyl methacrylate)-poly(L-aspartic acid) graft copolymer.[6] The transport experiment of sodium ion across the membrane from this copolymer suggested the formation of continuous phases of poly(L-aspartic acid) domain which functions as the transmembrane permeating pathway ("channel") for sodium ion and is regarded as the model of membrane protein. The channel-composing polypeptide segment in the membrane showed the pH-induced reversible conformational change which is considered to influence the ion permeability. The regulation of permeability of styrene glycol, a nonelectrolyte, induced by pH change[7] or calcium ion[8] in this membrane was observed.

In the present paper we will describe the permselectivity of sugars such as glucose (monosaccharide), lactose (disaccharide), and raffinose (trisaccharide) and its pH-induced regulation in the new biomembrane model prepared from poly(butyl methacrylate)-poly(L-glutamic acid) or -poly(L-aspartic acid) graft copolymer.

Experimental Section

Materials. *N*-Methyl-*N*-(4-vinylphenethyl)ethylenediamine (**1**) was synthesized by the addition reaction of *N*-methylethylenediamine with 1,4-divinylbenzene catalyzed by lithium alkylamide.[9] Butyl methacrylate (BMA) was distilled over calcium hydride. β-Benzyl L-aspartate *N*-carboxylic anhydride (BLA-NCA) and γ-benzyl L-glutamate *N*-carboxylic anhydride (BLG-NCA) were prepared by the reaction between β-benzyl L-aspartate or γ-benzyl L-glutamate, respectively, and a benzene solution of phosgene.[10] Styrene glycol, D-glucose, D-β-lactose, and D-raffinose were used as commercially available. Benzene, dichloromethane, hexane, and tetrahydrofuran (THF) were purified by the usual methods. The Britton-Robinson pH buffer solution[11] was prepared by mixing aqueous solutions of acetic acid, phosphoric acid, boric acid, and sodium hydroxide. The other chemicals used were of reagent grade.

Copolymerization of 1 and Butyl Methacrylate. Synthesis of Backbone Copolymer (2).[12] Radical copolymerization of **1** (0.5-0.7 mmol) and butyl methacrylate (31.7 mmol) was carried out in a sealed glass ampule at 45 °C with 2,2'-azobis(2,4-dimethylvaleronitrile) as initiator (0.06-0.07 mmol) and benzene as solvent (30 mL). After a definite



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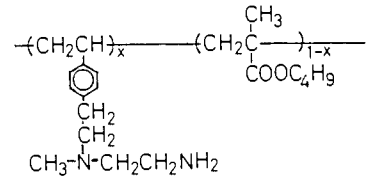

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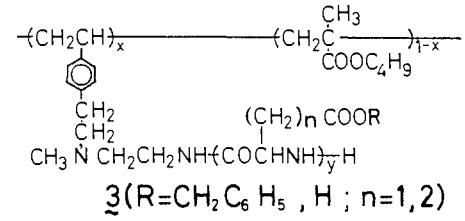

3 (R=$CH_2C_6H_5$, H; n=1,2)

period of time (14 h), the ampule was opened, and the contents were poured into a large excess of cold (2-3 °C) hexane (1000 mL). The precipitated polymer was redissolved in benzene and subjected to freeze-drying to give white solid in a 45-55% yield. Content of **1** in the copolymer **2** was determined from the area ratio of the signal of $-COOCH_2-$ of the butyl group to the signal of aromatic ring protons in

(1) Foster, D. L.; Boublik, M.; Kaback, H. R. *J. Biol. Chem.* **1983**, *258*, 31.
(2) Lehninger, A. L. *Biochemistry*, 2nd ed.: Worth Publishers, Inc.; New York, 1975; p 788.
(3) Solomon, A. K. *J. Gen. Physiol.* **1968**, *51*, 335; *Chem. Abstr.* **1968**, *69*, 24500p.
(4) Ortiz, M.; Lluch, M.; Ponz, F. *Rev. Esp. Fisiol.* **1979**, *35*, 239; *Chem. Abstr.* **1979**, *97*, 35029w.
(5) Osada, Y.; Takeuchi, Y. *Polym. J.* **1983**, *15*, 4.
(6) Maeda, M.; Kimura, M.; Hareyama, Y.; Inoue, S. *J. Am. Chem. Soc.* **1984**, *106*, 250.
(7) Higuchi, S.; Mozawa, T.; Maeda, M.; Inoue, S. *Macromolecules*, in press.
(8) Maeda, M.; Aoyama, M.; Inoue, S. *Makromol. Chem.*, in press.
(9) Maeda, M.; Nitadori, Y.; Tsuruta, T. *Makromol. Chem.* **1980**, *181*, 2251.
(10) Fuller, W. D.; Verlander, M. S.; Goodman, M. *Biopolymers* **1976**, *15*, 1869.
(11) Britton, H. T. S.; Robinson, R. A. *J. Chem. Soc.* **1931**, *458*, 1456.
(12) Kimura, M.; Egashira, T.; Nishimura, T.; Maeda, M.; Inoue, S. *Makromol. Chem.* **1982**, *183*, 1393.

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the ^1H NMR spectrum in $CDCl_3$. Molecular weight as determined by gel permeation chromatography calibrated with polystyrene in THF was $2-10 \times 10^4$.

Grafting Reaction. Attachment of the Polypeptide Branch. The synthesis of graft copolymer **3** (R = $CH_2C_6H_5$) was carried out by the polymerization of BLA-NCA or BLG-NCA initiated by the primary amino group of **2**.

A THF solution (8–15 mL) of BLA-NCA or BLG-NCA (0.5–0.8 g) was mixed with a dichloromethane or THF solution (20 mL) of copolymer (**2**) (0.5 g) at room temperature, and the mixture was stirred for 1 day. The reaction mixture was homogeneous throughout the reaction. Then the reaction mixture was poured into a large excess of cold (2–3 °C) hexane (1000 mL), and the precipitated polymer was filtered off and dried in vacuo. The average degree of polymerization of the poly(amino acid) chain grafted onto **2** was determined by ^1H NMR; since the content of **1** in **2** is known, the average number of amino acid residues in a side chain of the graft copolymer **3** (R = $CH_2C_6H_5$) was calculated by using the area ratio of the signal of $-COOCH_2-$ of the butyl group in the copolymer backbone to that of benzyl CH_2 in poly(β-benzyl L-aspartate) (**3**, R = $CH_2C_6H_5$, n = 1) or poly(γ-benzyl L-glutamate) branch (**3**, R = $CH_2C_6H_5$, n = 2), assuming the quantitative reaction of the primary amino group of **2**.

Preparation of Polymer Membrane. The graft copolymer dissolved in chloroform (about 5% solution) was cast on a glass plate (5.8 cm in diameter), and evaporation was carried out in a desiccator at room temperature for a day. Then the membrane was dried under reduced pressure for several hours. Membranes from the copolymer having the poly(γ-benzyl L-glutamate) branch could be detached from the glass plate in water more easily than the membranes with the poly(β-benzyl L-aspartate) branch. The membranes from the latter were prepared thicker than the former and were detached carefully in tepid water. The thickness of the membrane used in the permeation experiment was about 40 and 80 μm, respectively.

The membrane was fixed vertically in the center of a diaphragm cell (3 cm in diameter). In order to convert the benzyl ester group of the polypeptide branch to the carboxylate group, hydrolysis of the membrane was carried out by filling both sides of the cell with a ternary solvent from water, methanol, and 2-propanol (1:2:2, by volume) containing 0.5 wt % KOH for 16 h, followed by washing the membrane with the fresh ternary solvent. The debenzylation of the polypeptide branch is considered to be performed almost perfectly as estimated by the infrared spectra of the membrane and the amount of benzyl alcohol released from the membrane to the solution.[8] Thus, the membrane after hydrolysis is regarded to be composed of poly(butyl methacrylate)–poly(L-aspartic acid) (**3**, R = H, n = 1) or poly(butyl methacrylate)–poly(L-glutamic acid) (**3**, R = H, n = 2) graft copolymer.

Permeation. Both sides of the cell were filled with a buffer solution of a prescribed pH and kept for 2 days. After removal of the buffer solution, the membrane was washed with pure water several times and kept in pure water for about 1 h. Then, 50 cm^3 of aqueous solution containing styrene glycol and two or three kinds of sugar (initial concentration of respective substance, 0.1 M) was placed in one side of the cell and 50 cm^3 of pure water was placed in the other side. Both of the solutions were stirred slowly at 30 °C. At the time when about 3.5% of styrene glycol is permeated, as determined by ultraviolet spectrometry at 256 nm, the relative extent of permeation of sugars to styrene glycol was determined by ^1H NMR in D_2O:[13] styrene glycol, phenyl group, 7.3 ppm; glucose, H-1 of D-glucopyranose ring, α, 5.23 ppm, β, 4.65 ppm; lactose, H-1 of D-glucopyranose ring, α, 5.23 ppm, β, 4.65 ppm and H-1 of β-D-galactopyranose ring, 4.46 ppm; raffinose, H-1 of α-D-glucopyranose ring, 5.45 ppm. The extent of permeation for styrene glycol, lactose, and raffinose was calculated from the signal area of 7.3, 4.46, and 5.45 ppm, respectively. The extent of permeation for glucose was calculated from the signal area for α-anomer (5.23 ppm) by using the reported ratio of α- and β-anomers at room temperature,[14] since the β-anomer peak (4.65 ppm) falls on that of H_2O. In the case of the mixture of glucose and lactose, the amount of glucose was calculated by substracting the amount corresponding to lactose from the total amount of D-glucopyranose ring since lactose contains a D-glucopyranose ring and the chemical shift of its H-1 protons is the same as that of glucose.

Circular Dichroism (CD) Spectra. The graft copolymer (12 mg) having the poly(γ-benzyl L-glutamate) branch dissolved in chloroform (4 mL) was cast on a glass plate (5.8 cm in diameter), and evaporation was carried out to leave a thin film (ca. 3 μm in thickness), which was detached from the glass plate and was cut into a square. The square film was attached to a quartz plate (50 mm × 9 mm, 5 mm thick) by fixing both ends with wax and wrapping them by parafilm. Hydrolysis of the film was carried out by immersing the quartz plate attaching the film into a water–methanol–2-propanol (1:2:2, by volume) mixed solvent containing 0.5 wt % of KOH for 16 h. The CD spectrum of the film was measured by putting the quartz plate attaching the film into a quartz cell (path length, 10 mm) filled with pure water after immersing it into a buffer solution of a prescribed pH for 1 day.

Measurement. Ultraviolet spectrophotometry was done on a JASCO UVIDEC-1 recording spectrophotometer using a quartz cell of 10 mm optical path length. ^1H NMR spectra were obtained on a JEOL JNM-GX 400 FT NMR spectrometer operating at 400 MHz. Circular dichroism spectra were measured by using a JASCO J-500A spectropolarimeter.

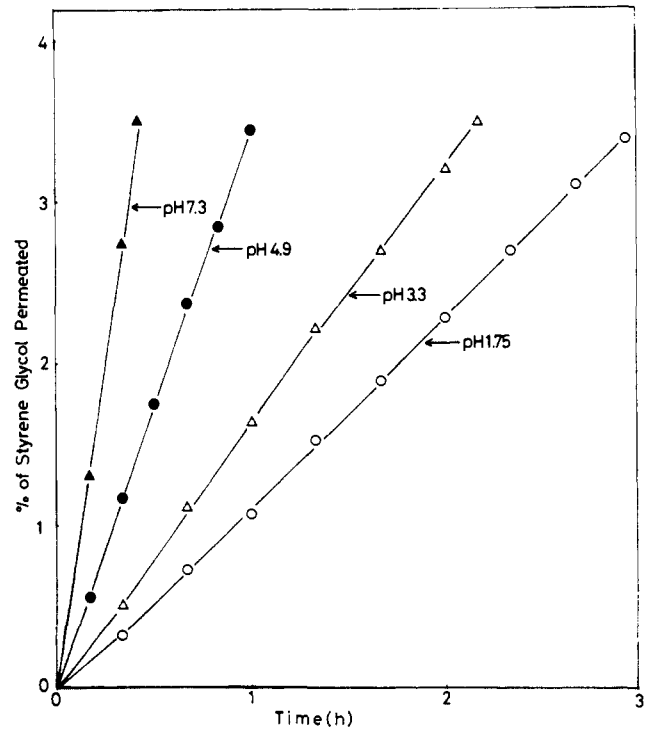

Figure 1. Time–transport curves of styrene glycol in the mixed system of styrene glycol, glucose, and raffinose across the PBMA-g-PLGA membrane after treatment at the prescribed pH.

Results and Discussion

Permeation across the Poly(butyl methacrylate)–Poly(L-glutamic acid) Graft Copolymer Membrane (PBMA-g-PLGA). Figure 1 illustrates representative examples of the permeation of styrene glycol in a mixed solution of styrene glycol, glucose, and raffinose across the poly(butyl methacrylate)–poly(L-glutamic acid) graft copolymer (PBMA-g-PLGA) membrane (**3**, R = H, n = 2; content of branch, x in **3**, 3.8 mol %; average degree of polymerization of branch, y, 16) after being kept at a prescribed pH for 2 days. From Figure 1, the rate of permeation of styrene glycol was estimated as the amount of the substrate transported in a unit time (Figure 2). The sigmoidal shape of the rate–pH profile demonstrates the significant change in permeability of the membrane in the region of pH around 4.5. Almost the same rate–pH profile was obtained when pH was changed from high to low (○) and from low to high (●). Similar rate–pH profiles were observed also for sugars. In the region of high pH, the carboxyl groups are considered dissociated to carboxylate so that poly(L-glutamic acid) chains expand into random coil conformation and the hydrophilicity of the permeating domains increases, resulting in the high permeability. In the region of low pH, the permeability decreases remarkably probably due to the contraction of polypeptide chains into α-helical conformation and the decrease in the hydrophilicity of the permeating domains corresponding to the protonation of the carboxylate groups.

Circular Dichroism (CD) Spectra of the Film. In fact, pH-dependent conformational change of poly(amino acid) chains of the graft copolymer in the film was observed by circular dichroism (CD) studies.

(13) Bruyn, A.; Anteunis, M.; Beeumen, J. V. *Bull. Soc. Chim. Belg.* **1977**, *84*, 259.
(14) Angyal, S. J. *Aust. J. Chem.* **1968**, *21*, 2737.

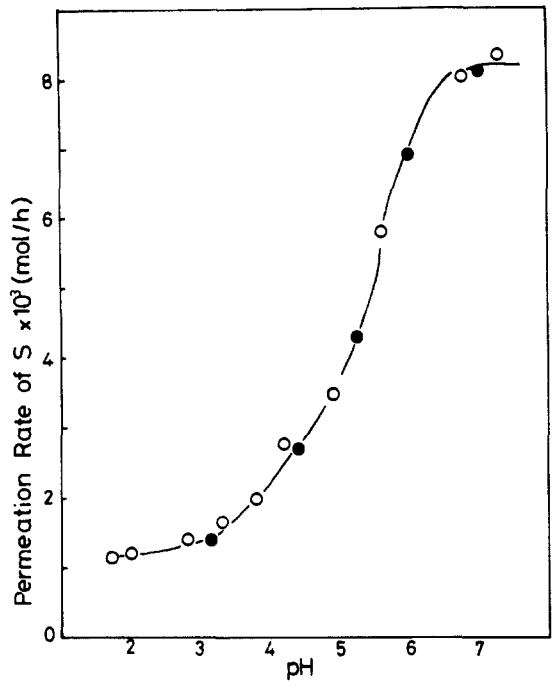

Figure 2. Permeation rate of styrene glycol (*S*) in the mixed system of styrene glycol, glucose, and raffinose across the PBMA-g-PLGA membrane; change in pH from high to low (○) and from low to high (●).

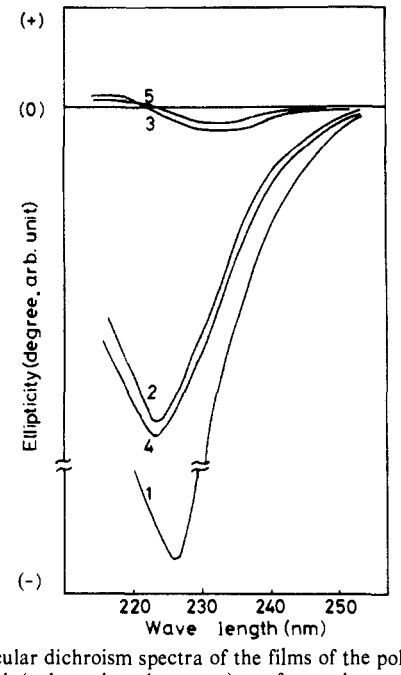

Figure 3. Circular dichroism spectra of the films of the poly(butyl methacrylate)–poly(γ-benzyl L-glutamate) graft copolymer (before hydrolysis) and –poly(L-glutamic acid) graft copolymer (after hydrolysis): 1, before hydrolysis; 2, after hydrolysis, at pH 2; 3, after hydrolysis, at pH 7; 4, after hydrolysis, at pH 2; 5, after hydrolysis, at pH 7. Numbers represent the order of CD measurement.

Figure 3 shows the CD spectra of the film of the poly(butyl methacrylate)–poly(γ-benzyl L-glutamate) graft copolymer (**3**, $n = 2$, $x = 3.8$ mol %, $y = 16$) before and after hydrolysis. 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 take mainly right-handed α-helical conformation, in a similar manner to the homopolymer of γ-benzyl L-glutamate in the film.[15]

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 relative maximum value of ellipticity at pH 2 of hydrolyzed film was ca. 30% of that before hydrolysis. The negative peak at 223 nm indicates the formation of right-handed α-helix, in a similar manner to poly(L-glutamic acid) in acidic aqueous solution[16] and in the film.[17] Although α-helix is known to show another band at 208 nm, the absorption of ester groups of main chain and amide groups of side chain was too strong to observe the CD curves below ca. 215 nm even for the membrane as thin as possible. Poly(L-glutamic acid) in the β-conformation is reported to have the minimum at 226 nm,[18] but Figure 3 shows no trough in this region. On the other hand, the shift of the 222-nm band toward 225 nm and flattening of the 208-nm band have been indicated as characteristic of the aggregate of α-helices of poly(L-glutamic acid).[19,20] Thus, the poly(L-glutamic acid) chain in the present membrane is considered to be mainly in the α-helix or aggregate of α-helices, although minor existence of β-conformation may not be excluded.

The CD spectrum of the film after pretreatment with a buffer solution of pH 7 showed a pronounced decrease in the intensity at 223 nm, which indicates the transformation from α-helical to random coil form of the poly(L-glutamic acid) chain in the film. This behavior is reversible; upon lowering the pH from 7 to 2 and increasing it from 2 to 7, almost the same CD curve reappeared at the same pH, respectively, as shown in Figure 3. When the branch is poly(L-aspartic acid), a similar change in CD spectra with pH was observed.[6]

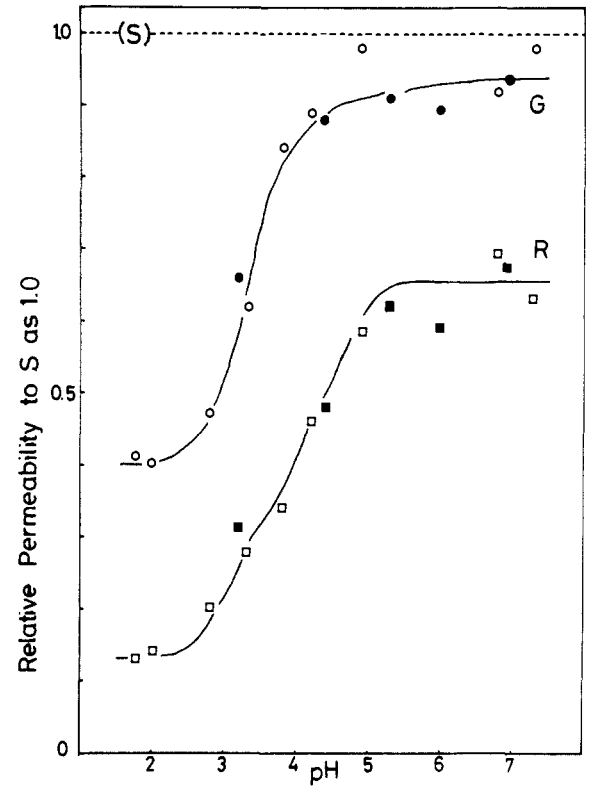

Figure 4. Relative permeability of glucose (G) and raffinose (R) to styrene glycol (S) as the reference in the mixed system of (S), (G), and (R) across the PBMA-g-PLGA membrane; change in pH from high to low (○, □) and from low to high (●, ■).

Relative Permeability of Sugars. Figure 4 illustrates the relative permeability of sugars to styrene glycol as the reference. Throughout the region of pH examined, the permeation rates were

(15) Sederel, W. L.; Bantjes, A.; Feijen, J. *Biopolymers* **1980**, *19*, 1603.
(16) Holzwarth, G.; Doty, P. *J. Am. Chem. Soc.* **1965**, *87*, 218.
(17) Fasman, G. D.; Hoving, H.; Timasheff, S. N. *Biochemistry* **1970**, *9*, 3316.
(18) Itoh, K.; Foxman, B. M.; Fasman, G. D. *Biopolymers* **1976**, *15*, 419.
(19) Maeda, H.; Kato, H.; Ikeda, S. *Biopolymers* **1984**, *23*, 1333.
(20) Pieroni, O.; Fissi, A.; Houben, J. L.; Ciardelli, F. *J. Am. Chem. Soc.* **1985**, *107*, 2990.

in the order styrene glycol > glucose > raffinose.

From Figure 4, the relative permeation rate of raffinose (trisaccharide) to glucose (monosaccharide) was found to be much more diminished in the region of low pH (relative rate, 0.34 at pH 2) than in high pH (0.68 at pH 7). Assuming that the hydrophilicities of glucose and raffinose are almost the same, the remarkable decrease in relative permeability of raffinose in the region of low pH is probably due to the structural change of molecular assembly of polypeptide in permeating domains. In the region of high pH, the polypeptide chain is in the random coil conformation, and the permeating domains composed of the polypeptide assembly become relatively loose. Thus, the permeabilities of raffinose and glucose are less different in the high pH region than in the low pH region. In the region of low pH, the polypeptide chain is mainly in the rigid α-helical conformation and permeating domains become relatively tight compared with those in the region of high pH. Thus, relative permeability of raffinose with larger molecular size to glucose with smaller size is diminished in the low pH region.

The relative permeability of sugars to styrene glycol is also remarkably diminished in the region of low pH. The difference in permeability between sugars and styrene glycol is considered not only due to conformational change but also due to the hydrophilicity change of permeating domains. In the region of low pH, hydrophobicity of the permeating domains increases due to the protonation of the carboxylate group so that it becomes relatively difficult for sugars to permeate across the membrane since sugars are less hydrophobic compared with styrene glycol.

Permeation across the Poly(butyl methacrylate)–Poly(L-aspartic acid) Graft Copolymer Membrane (PBMA-g-PLAA). Permeation across the poly(butyl methacrylate)–poly(L-aspartic acid) graft copolymer (PBMA-g-PLAA) membrane, with the content of the branch (x in **3**, R = H, n = 1) 4.8 mol % and the average degree of polymerization of the branch (y) of 21, was examined in the mixed aqueous solution of styrene glycol, glucose, lactose, and raffinose. Similarly to the case of the poly(butyl methacrylate)–poly(L-glutamic acid) graft copolymer membrane, the permeation rate of styrene glycol exhibited a significant change in the region of pH around 4.5, the difference being about 100 times betwen pH 2 and 7, and the relative permeability of sugars to styrene glycol was diminished in the region of low pH.

Comparing the permeabilities of lactose and raffinose with the permeability of glucose, the decrease in relative permeabilities of lactose and raffinose with larger molecular size is observed in the low pH region (Figure 5). The permeability of glucose (monosaccharide) to raffinose (trisaccharide) is two times at pH 7 and ten times at pH 2.

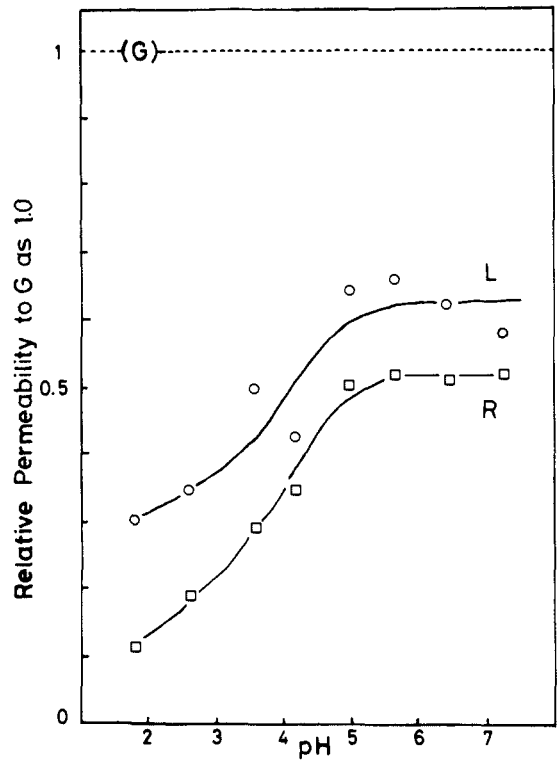

Figure 5. Relative permeability of lactose (L, ○) and raffinose (R, □) to glucose (G) as the reference in the mixed system of styrene glycol, (G), (L), and (R) across the PBMA-g-PLAA membrane; change in pH from high to low.

Conclusion

Permeability of sugars and styrene glycol in their mixtures can be regulated by the change in pH for the polymer membrane from poly(butyl methacrylate)–poly(L-glutamic acid) or –poly(L-aspartic acid) graft copolymer. This is considered due to the change in hydrophobicity–philicity of permeating domains and the structural change of permeating domains caused by the conformational change of the polypeptide chain brought about by the reversible dissociation of the carboxyl group. This membrane may be regarded to recognize the hydrophobicity–philicity and molecular size of permeating substances. In this respect, this membrane mimics some characteristics of the transport system of sugars in the biomembrane.